

## **Partnership Projects with NOAA National Centers for Coastal Ocean Science**



## **Assessing Coral Health in the Florida Keys National Marine Sanctuary Using a Molecular Biomarker System**

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### ***Goals***

We have designed an integrated Cellular Diagnostic System (CDS) to diagnose whether an organism is stressed and to identify the likely stressor(s) (e.g., heat stress, pesticides, and pathogens). Our goals are to: (1) use the CDS to characterize the health of a coral reef ecosystem in the Florida Keys; (2) verify that the CDS can detect and characterize subtle and chronic effects of environmental stressors on this ecosystem; (3) determine if point-source pollutants or global climate changes (e.g., increased ocean temperatures or UV-B radiation) are stressing coral reef ecosystems; (4) compare the precision, sensitivity, and prognostic capabilities of the CDS to those of traditional measures of ecosystem health, and (5) encourage participation and understanding of the general public, scientific, industrial, and managerial communities in using marine biotechnologies to assess and manage the health of coral reef ecosystems.

### ***Methods***

The methods being employed range from established protocols for community-scale assessment (i.e., the AGRRA protocol of Ginsburg et al. 2000), foraminiferal condition (i.e., Hallock Muller et al. 1995), and CDS analysis (previously known as MBS) (Downs et al. 2000, 2001), to methods adapted to monitor coral lesions and sedimentation.

#### ***Cellular Diagnostics***

The Cellular Diagnostic System is an ELISA-based assay system, specifically used to measure changes in cellular parameters, and allows assessment of cellular-physiological condition, monitoring of cellular stress responses, identification of putative stressors, and provision of a prognosis. Cellular diagnostics uses a systematic approach to quantifying cellular and biochemical responses of defined biomarkers of exposure, effect, and susceptibility based on their functionality within a cell and integrate, or profile, those responses into a diagnosis and subsequently a prognosis. Knowledge of biomarker function helps describe how alterations in the behavior of a single cellular parameter or set of cellular parameters (biomarkers) may affect overall cellular operation or performance (Downs 2004). We use this information to distinguish cellular diagnostics from biomarkers, which are biological response elements without known functional association.

The cell is a dynamic system comprised of both macro- and micro-structures and processes. Many of these sub-cellular processes are key metabolic pathways and cellular structural

components that are essential for maintaining cellular operations, homeostasis, and cell functionality. These cellular metabolic pathways and structural operations can be divided into categories or sub-systems of cellular integrity and function, which can be further defined by discrete parameters (Table 1). The behavior of these components and processes defines the physiological condition of the cell. Stressors affect ecosystems by overwhelming allostatic defenses (i.e., the continuous process of adaptation experienced by the host in the face of potentially stressful challenges; Seeman et al. 1997) at lower levels of the biological hierarchy — specifically, molecular, cellular, and organism-level homeostatic processes. As the *allostatic load* (i.e., the wear and tear experienced as a result of repeated cycles of allostasis; McEwen et al. 2003) increases there is an overall accumulation of negative effects of adaptation to various challenges and adverse environments -- superimposed on such things as genetic predisposition and development. Thus stressors (allostatic load) reduce individual fitness, alter demographic patterns, and affect the structure, function, and resilience of coral reef communities. Changes in cellular and molecular parameters may precede ecosystem-level responses to chronic stress by days, months, or years. Measuring changes in these cellular parameters allows: (1) determination of cellular-physiological condition of an individual or population; (2) identification of putative stressors, either by direct measurement of the stressor or by profiling stressor-specific effects; and (3) forecasting higher-order behavior based on an understanding of cellular-level processes (Downs 2004). Cellular diagnostics provides a new approach to health assessment, though its fundamental tenets are rooted on concepts and methodologies central to medical diagnostics and epidemiology.

**Table 1. Categories and Parameters of Cellular Integrity and Homeostasis**

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**Genomic integrity** – the ability of the genomic process to maintain a functional state. Parameter assayed in this project: Ogg1-nuclear.

**Protein metabolic condition** – the process of protein synthesis, protein maturation, and protein degradation. Parameters assayed were: Hsp 60 (cnidarian and dinoflagellate), Grp75 (Mortalin), Hsp70 (cnidarian and dinoflagellate), Hsp90, ubiquitin, and ubiquitin activase.

**Xenobiotic detoxification** – the process of preventing or reducing the adverse (toxic) effects of exposure to a xenobiotic. Parameters assayed were: glutathione-s-transferase, MDR, CYP P450 2 class, CYP P450 3 class, and CYP P450 6 class.

**Metabolic integrity** – the process of a cell in maintaining a differentiated state from its environment and is the product of sub-processes or ‘metabolic’ pathways. Parameters assayed were: (20) ferrochelatase, Protoporphyrinogen IX oxidase, heme oxygenase 1, mitochondrial small heat-shock protein, and chloroplast small heat shock protein.

**Oxidative damage and response** - the process of maintaining a viable condition in an oxygen-laden environment. Parameters assayed were protein carbonyl, catalase, glutathione peroxidase, Mn superoxide dismutase, and Cu/Zn superoxide dismutase.

**Endocrine modulation** – parameters indicative of endocrine disruption include aspects that focus on classical endocrine systems besides changes in reproductive structure. Endocrine/reproduction parameters included vitellogenin level in males.

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### ***Lesion Regeneration***

Coral lesions (i.e., partial mortality) of tagged corals (*Montastraea annularis* complex) were monitored quarterly (March/April, June, August/September, and October/November) in 2001 and 2002 and again in February 2003. A lesion was defined as an area on the colony with no live coral tissue. Lesions created by the biomarker sampling were approximately 2 cm<sup>2</sup> and should regenerate under non-stressful conditions (Meesters et al. 1997). To monitor lesions, each lesion was photographed using a Nikonos V camera with a close-up adapter. Photographs were scanned to digital images, and the area and perimeter of the lesions were calculated using image analysis software. These data will be used to determine whether correlations exist between coral lesion regeneration rates and molecular-scale responses of individual coral colonies providing quantitative indicators of stresses. Data on water temperature, nutrient levels, foraminiferal populations, and sedimentation will then enable us to determine if these factors correlate with changes in lesions and with stress levels quantified by the CDS. The hypothesis being tested is that a coral, which the CDS indicates to be more stressed, will be less likely to regenerate than a coral that CDS indicates to be less stressed.

### ***Findings to Date***

Our results indicate that this technology can be used to characterize coral health in defined areas of the Florida Keys (Goal 1), distinguish between global-level stressors (e.g., El Niño/La Niña effects) and local-level stressors (e.g., agricultural runoff) (Goals 2 and 3), and help predict the condition of corals several months before more obvious symptoms appear (e.g., coral bleaching or coral death) (Goals 3 and 4). Additionally, comparisons of coral lesion healing with biomarker response have shown significant correlations between the level of biomarker response (representative of the cellular physiological status) and measures traditionally used to assess ecosystem health (Goal 4). These results support our hypothesis that a coral that the CDS indicates to be more stressed will be less likely to regenerate than a coral that CDS indicates to be less stressed.

To build on results from 1999 and 2000, a two-year study (2001-2002) was conducted to test “proof of concept” for the efficacy of CDS in assessing coral reef ecosystem health. This study included two sites in Biscayne National Park (BNP) and six sites in the Florida Keys National Marine Sanctuary (FKNMS). Our design expanded sampling from just coral tissue (*M. annularis* complex), to include snails (*Coralliophila abbreviata*) and fish (white grunt: *Haemulon plumieri* and bicolor damselfish: *Stegastes partitus*). We also included a coralline green alga, *Halimeda opuntia*, initially; however, technical difficulties with protein extracts prevented analysis.

The cumulative results for 2001 and 2002 showed that this technology can be used to detect changes in the physiological condition of corals, snails, and fish and provided evidence for the type of stressor(s) that were responsible for these changes. Also included were traditional measures of coral ecosystem health, foraminiferal condition indexing, and an intermediate biomarker that integrates a number of cellular processes (lesion regeneration). These parameters were supportive and correlated with measures obtained from cellular physiological parameters. In general, our findings showed evidence for different stressors at different locations at different times and evidence that multiple stressors were responsible for physiological responses being observed. In some locations this meant healthy corals able to cope with the stressors and in other locations, coral death (loss of all living tissue). More specifically, white grunt profiles showed

that these fish were experiencing xenobiotic exposures, particularly at Alina's Reef in BNP, while in 2002 at both Alina's Reef and East Bache Shoals these fish exhibited profiles that supported an endocrine disruption occurring as a result of toxicant exposures. A profile supporting endocrine disruptor exposure as well as oxidative stress was exhibited in bicolor damselfish, particularly in 2002; however, fish with these profiles were not limited to BNP. Snails appeared to be a good indicator of coral reef health. They showed little evidence of stress in 2001 while in 2002-2003 their profiles at our 6-m depth site in the FKNMS were consistent with exposure to an endocrine disruptor. In general the coral, *Montastraea annularis*, at Algae Reef, White Banks Dry Rocks, and our Key Largo 3-m depth sites, appeared to be physiologically stable; however, it did show increased stress compared to coral located at Dry Tortugas. In contrast, corals at the two reefs in BNP (Alina's Reef and East Bache Shoals) and at the 6-, 9-, and 18-m depth sites (Sites 2-4) in the FKNMS showed elevated levels of stress and overall poor physiological health. By February 2003, colonies at the 9-m site showed heavy algal overgrowth and two colonies with very little live tissue remaining. At the 18-m site colonies appeared to be dying and one colony had lost all living tissue. The trend over the two years of sampling was a general decline at the 9- and 18-m sites (Sites 3 and 4) with at least one colony completely dying.

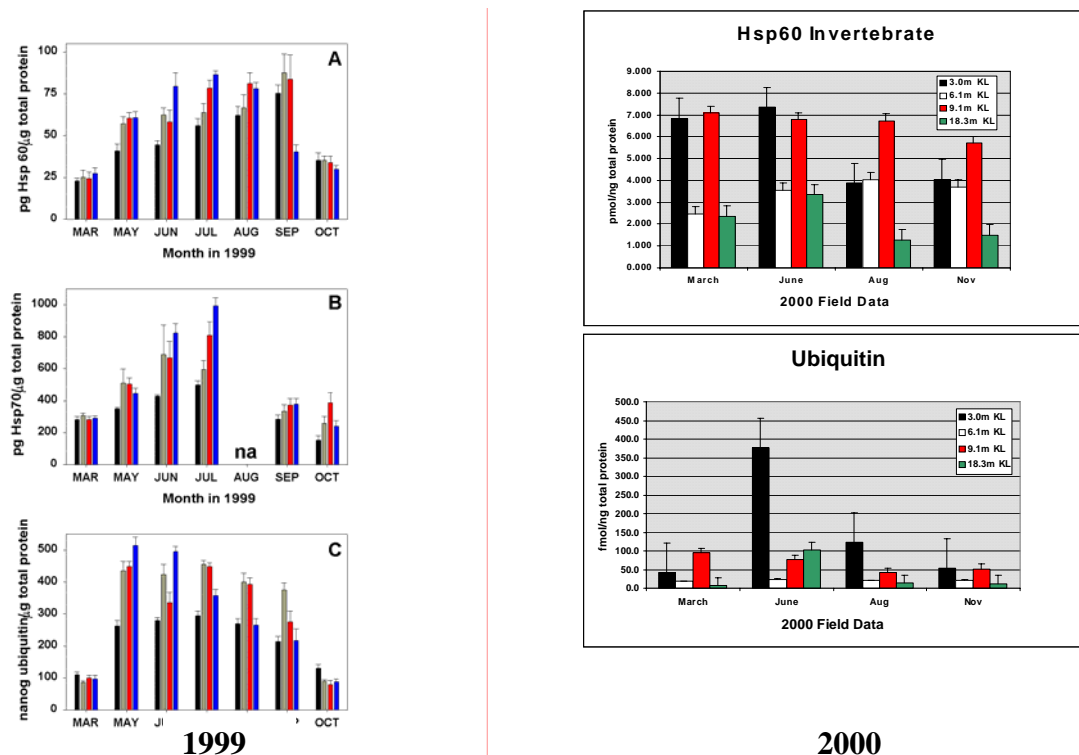
In general, responses varied throughout the year with the winter months, late October through March, appearing more stressful, with summers less stressful. This is in contrast to 1999, which was a year with unusually high sea surface temperatures. The profiles obtained from 2000 through February 2003, we believe, were reflective of local conditions, and the physiological profiles of each of these trophic levels provided evidence of multiple anthropogenic stressors impacting coral reef ecosystem health.

Our findings were presented (Goal 5) to constituent groups in March 2002 and January 2004 including state and federal resource managers, coral biologists, and representatives from the general public who have been encouraged to offer input and collaborations on this and related projects.

#### *Results and Data*

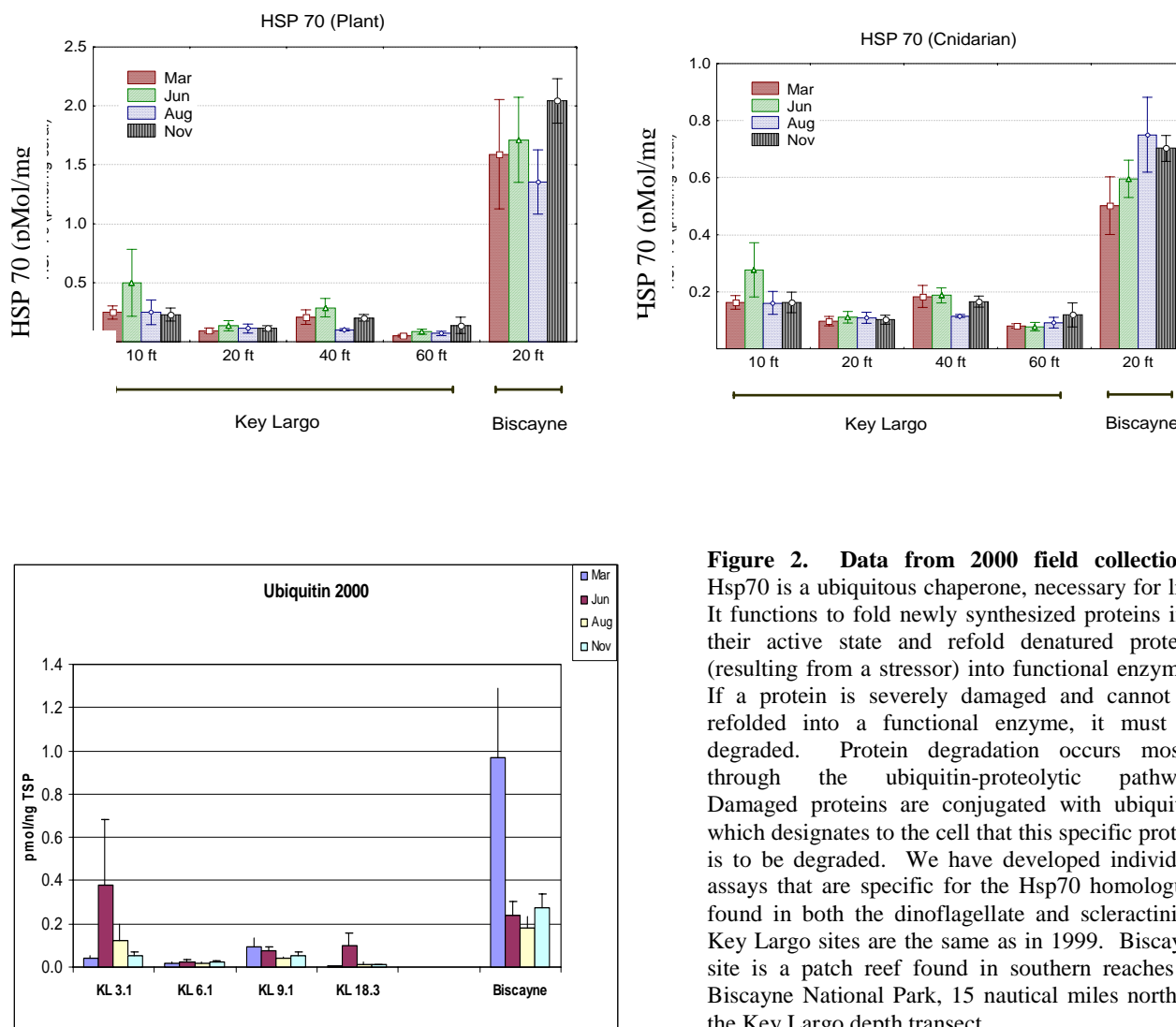
##### *Objectives 1-3*

The Cellular Diagnostic System (CDS) was developed to focus a comprehensive array of biotechnologies on the diagnosis of a variety of ecosystem health issues; however, for this study we specifically tailored it to coral physiological health and discerning the causes of coral reef system declines (Downs et al. 2000; Woodley et al. 2001). In our initial studies, we specifically applied the CDS to corals in the Florida Keys and demonstrated that the CDS could distinguish whether a local coral population of *Montastraea annularis* was being stressed by a global stressor (e.g., high sea-surface temperatures; Fig. 1) or by a stressor that was local in nature (Fig. 2). In conjunction with other technologies and monitoring methods, this biotechnology was able to identify potential stressor(s) responsible for the decline (Fig. 3). The CDS also possessed the ability to predict the progression of a health condition based on key diagnostic markers (Fig. 4). In 2001 and 2002, we expanded our studies to address whether the CDS could also be used to assess the health of other reef organisms (snails and fish) and whether CDS evaluation of members of different trophic levels within a coral reef ecosystem could be used to assess overall ecosystem health.



**Figure 1.** The same coral colonies from four sites at different depths were sampled on a monthly basis in 1999 and a quarterly basis in 2000. Hsp60 reflects chaperonin levels in the scleractinian; mean concentrations varied significantly with depth, month, and the depth x month interaction in 1999 (repeated measures MANOVAs: all  $F > 2.56$ ,  $P < 0.02$ ). Ubiquitin levels reflect the rate of protein degradation, which varied significantly with depth, month, and the depth x month interaction (repeated measures MANOVA: all  $F > 8.80$ ,  $P < 0.0001$ ). Bars show untransformed mean ( $\pm 1$  SE) biomarker concentrations at each depth: for 1999 panel, black = 3.0 m, grey = 6.1 m, red = 9.1 m, and blue = 18.3 m. Sites are from a four-mile-long transect off the eastern shore of Key Largo.

In Fig. 1, *M. annularis* scleractinian Hsp60 and ubiquitin data from the 1999 sampling project can be diagnostically interpreted as follows: the corals, at all four depths, were experiencing a protein-denaturing stress. This was indicated by a positive correlation between increased ubiquitin levels (a key component of a pathway for degrading 80% of the proteins in the cell) and abnormally high sea surface temperatures that peaked in the months of July and August (Downs et al. in review a). Hsp60 (for description of function, see Downs et al. 2000) data in 1999 corroborated this diagnostic interpretation. Though the extent of cellular damage differed significantly with depth, the data supported the argument that coral cellular damage at all four sites was the result of a global stressor (La Niña sea-surface temperature effects).

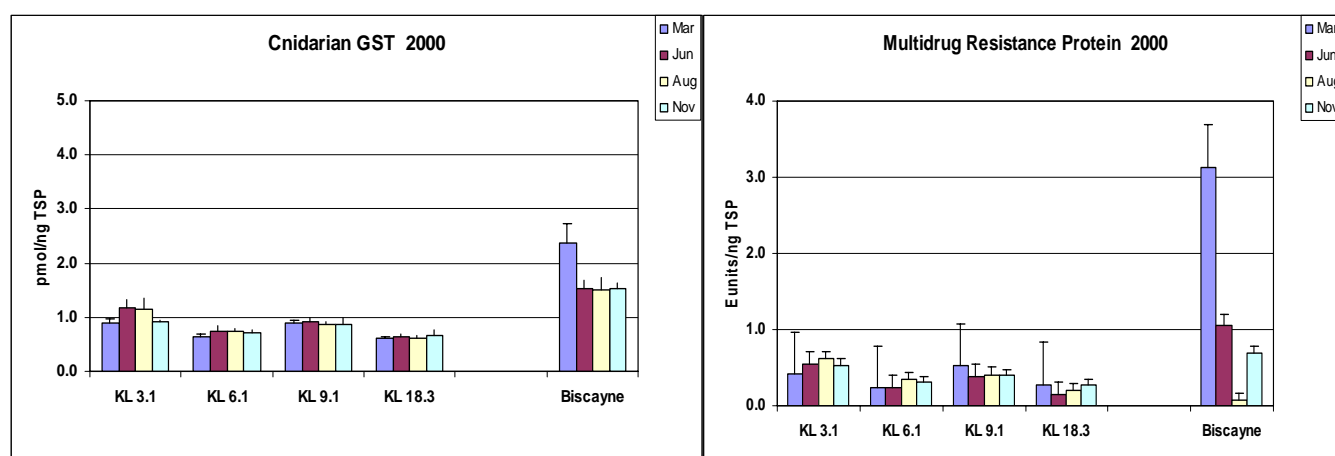


**Figure 2. Data from 2000 field collections.** Hsp70 is a ubiquitous chaperone, necessary for life. It functions to fold newly synthesized proteins into their active state and refold denatured proteins (resulting from a stressor) into functional enzymes. If a protein is severely damaged and cannot be refolded into a functional enzyme, it must be degraded. Protein degradation occurs mostly through the ubiquitin-proteolytic pathway. Damaged proteins are conjugated with ubiquitin, which designates to the cell that this specific protein is to be degraded. We have developed individual assays that are specific for the Hsp70 homologues found in both the dinoflagellate and scleractinian. Key Largo sites are the same as in 1999. Biscayne site is a patch reef found in southern reaches of Biscayne National Park, 15 nautical miles north of the Key Largo depth transect.

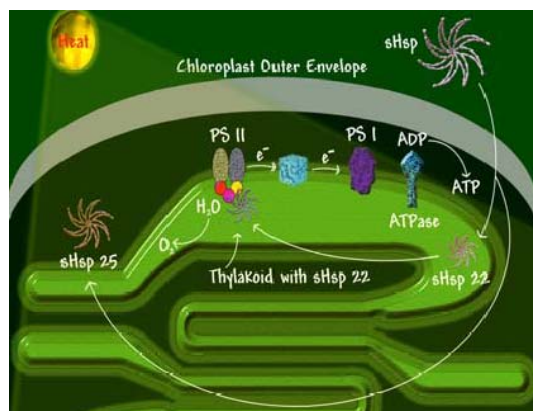
In 2000, the patterns of both parameters were radically different than those observed in 1999 and were not correlated with sea-surface temperatures (Woodley et al. in prep.). In March 2000, corals at 3-m depth were not experiencing a protein-denaturing cellular condition; however, they were experiencing non-adverse changes in mitochondrial function. In June 2000, corals at the 3-m site showed signs of cellular stress, which adversely affected mitochondrial function. These diagnostic interpretations for both 1999 and 2000 were corroborated by other diagnostic biomarker data. In summary, the cellular stress experienced by corals at all four sites in 1999 was the result of a global stressor as opposed to a local stressor at the 3- and 9-m sites in 2000 (and the stressor was different for these two 2000 sites – Woodley et al. in prep.). In 2000, using only three diagnostic markers (out of 24 biomarkers assayed for each coral sample), we could determine that a coral reef site in Biscayne National Park (BNP) was experiencing a severe cellular stress that was most likely generated by an electrophilically modifiable xenobiotic (e.g., a fungicide: an organometalloid, endosulfan) (Fig. 2 and 3). The extremely high level of ubiquitin indicated high rates of protein turnover. This interpretation was corroborated by five other cellular biomarkers. The level of ubiquitin in March 2000 at the BNP site has been



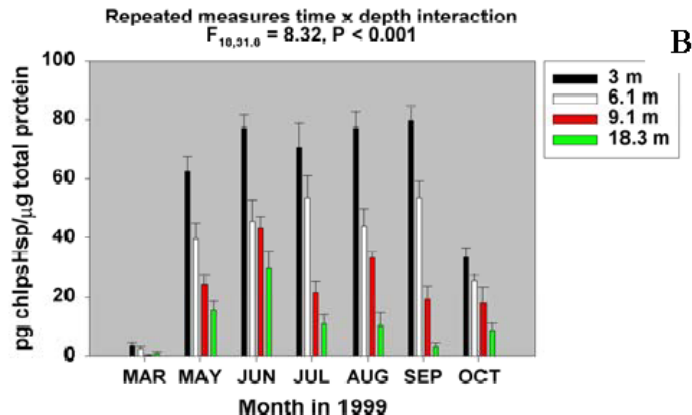
suggested to be near the maximal threshold capacity for this coral species – massive cellular deterioration was beginning to occur and coral death could be predicted (Downs and Woodley in prep.). In August 2000, significant and punctuated coral coverage loss at the BNP site was observed – no observable coral coverage loss was observed in March 2000. This partially unidentified stressor adversely affected both scleractinian and dinoflagellate cellular physiology (Fig. 2). Data presented in Fig. 3 can be interpreted as follows: corals at the BNP site were responding to a xenobiotic stressor and the response pathway included a mono-oxygenase catalytic reaction at the site of olefinic double bonds of the xenobiotic, the conjugation of glutathione to the xenobiotic by glutathione-s-transferase, and cellular exclusion of the GSH-conjugated xenobiotic by a P-glycoprotein 140/160 pump action (a.k.a. MDR: multi-drug resistance gene) (Woodley et al. in prep.; Downs et al. in prep. b; not all data shown for this interpretation).



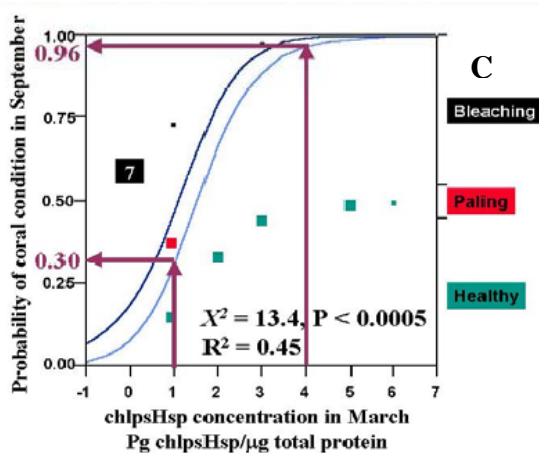
**Figure 3.** Data from 2000 field collections. GST Invertebrate = scleractinian homologues of glutathione-S-transferase. GST is an enzyme that will conjugate a xenobiotic with reduced glutathione so that the xenobiotic can easily be managed by the cell. MDR = P-glycoprotein 160, a member of the ABC family of proteins that is up-regulated when an organism has been exposed to specific classes of xenobiotics. Its function is to detoxify the cell of xenobiotics by pumping these xenobiotics out of the organism. Site locations are the same as described in Fig. 2.



A



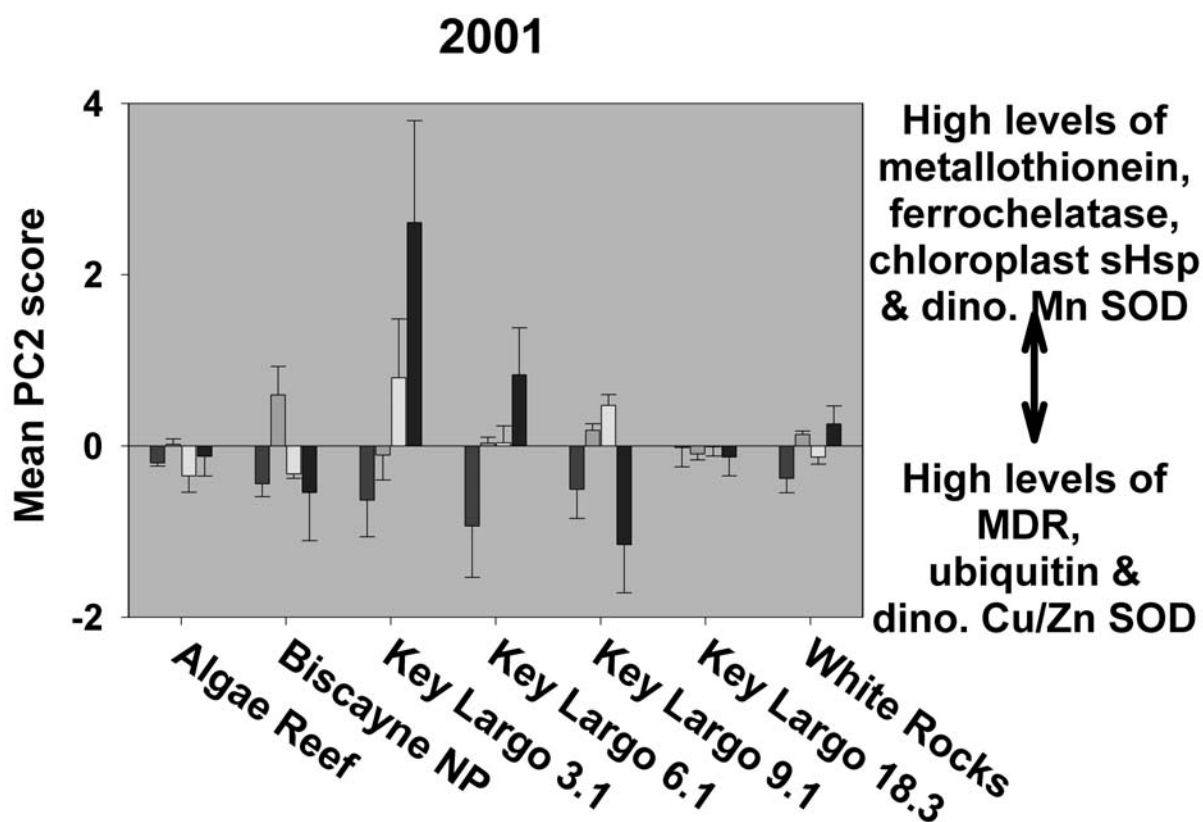
B



C

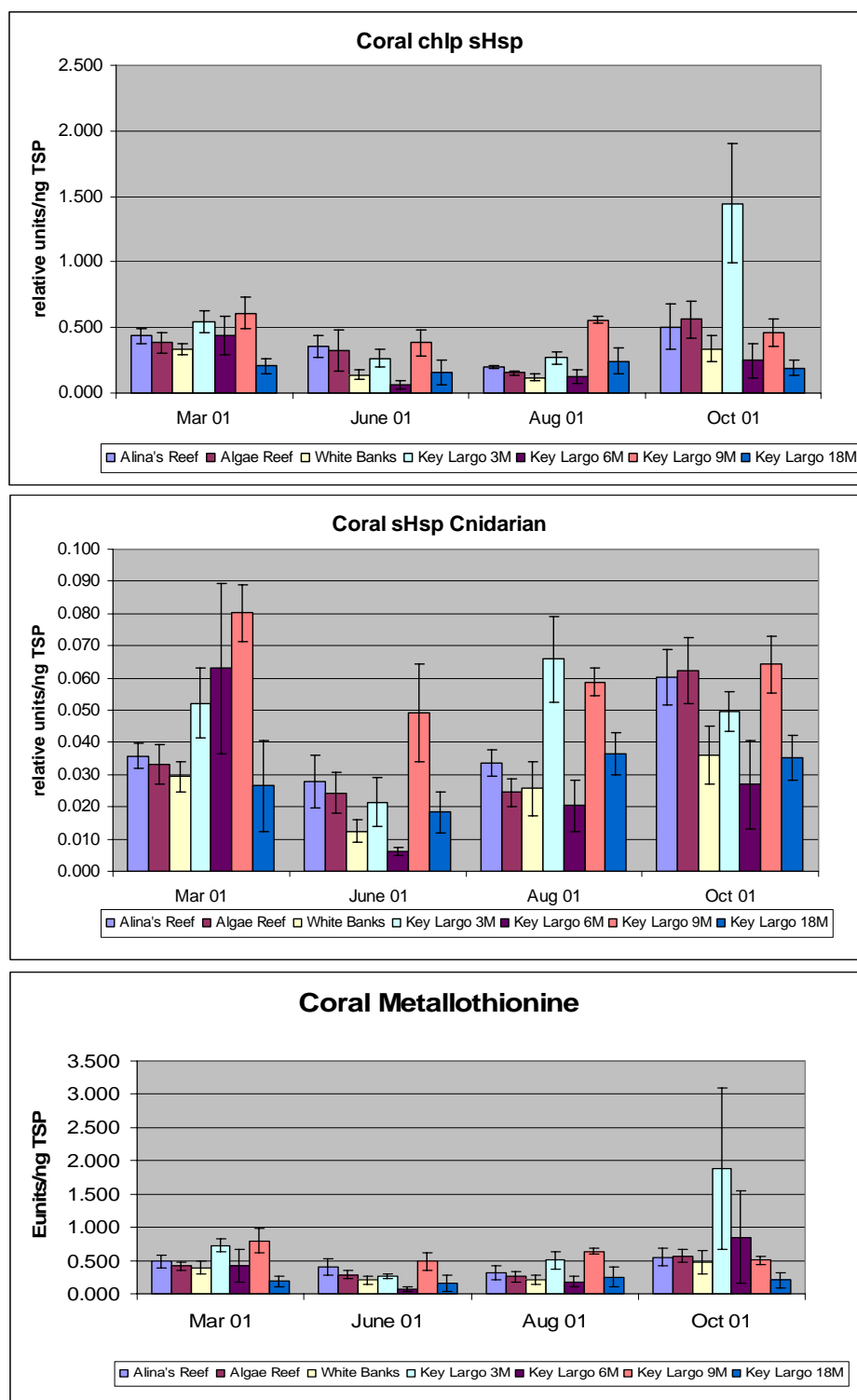
**Figure 4.** Panel A – Function of the chloroplast small heat-shock protein (ChlpsHsp). This protein is only induced when photosystem II is being damaged. It is a major adaptation of photosynthesis against heat stress, oxidative stress, and photoinhibition (Downs et al. 1999a, b). Panel B – Levels of ChlpsHsp in dinoflagellate of *M. annularis*. Coral samples and sampling scheme the same as in Fig. 2. (Downs et al. in review). Panel C- Logistic regression analysis of probability of the level of chlpsHsp in March to predict coral bleaching in September when sea surface temperatures reached 31°C in August (Fauth et al. in prep.).

In 2001 we continued to detect responses that indicated local stressors. Using a principal component analysis (Fig. 5), the following patterns emerged. The Key Largo 3-m site differed greatly from all the other six sites. The large upward spike in October suggested that a response to metals dominated over a xenobiotic response (data not shown). The Key Largo 6-m site had a similar profile, only not as pronounced. One possible explanation is that sedimentation and runoff may have contributed to these responses. Although we cannot unambiguously identify the specific stressor at this time, we know that it dramatically affected the algal component of the coral, as indicated by markers specific for the chloroplasts and algal mitochondria, and that it was likely associated with runoff and rainfall events. The effect of sedimentation will be tested using sedimentation data collected from sediment traps. In addition, except for the most offshore site, all sites had negative PC2 scores in March, suggesting that responses to a xenobiotic predominated over a metal stress. Again, one possible explanation is pesticide runoff. This will be further investigated by examining whether correlations exist between cellular diagnostic parameter levels and water chemistry parameters (chlorophyll a and/or pigments).

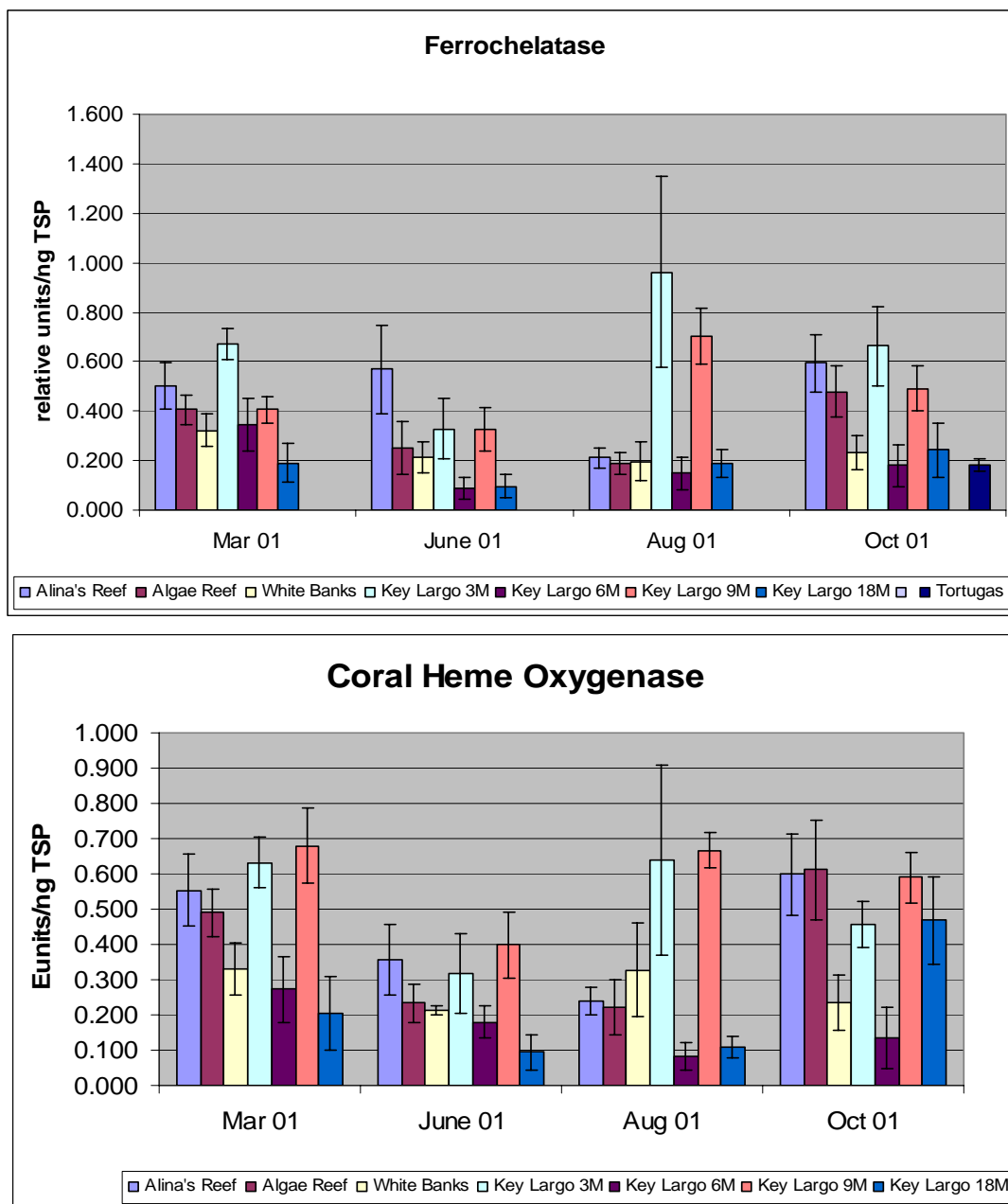


**Figure 5.** Results of the principal component analysis conducted on data from seven sites during different sampling periods; red = March; green = June; yellow = August; blue = November).

## Metabolic Condition

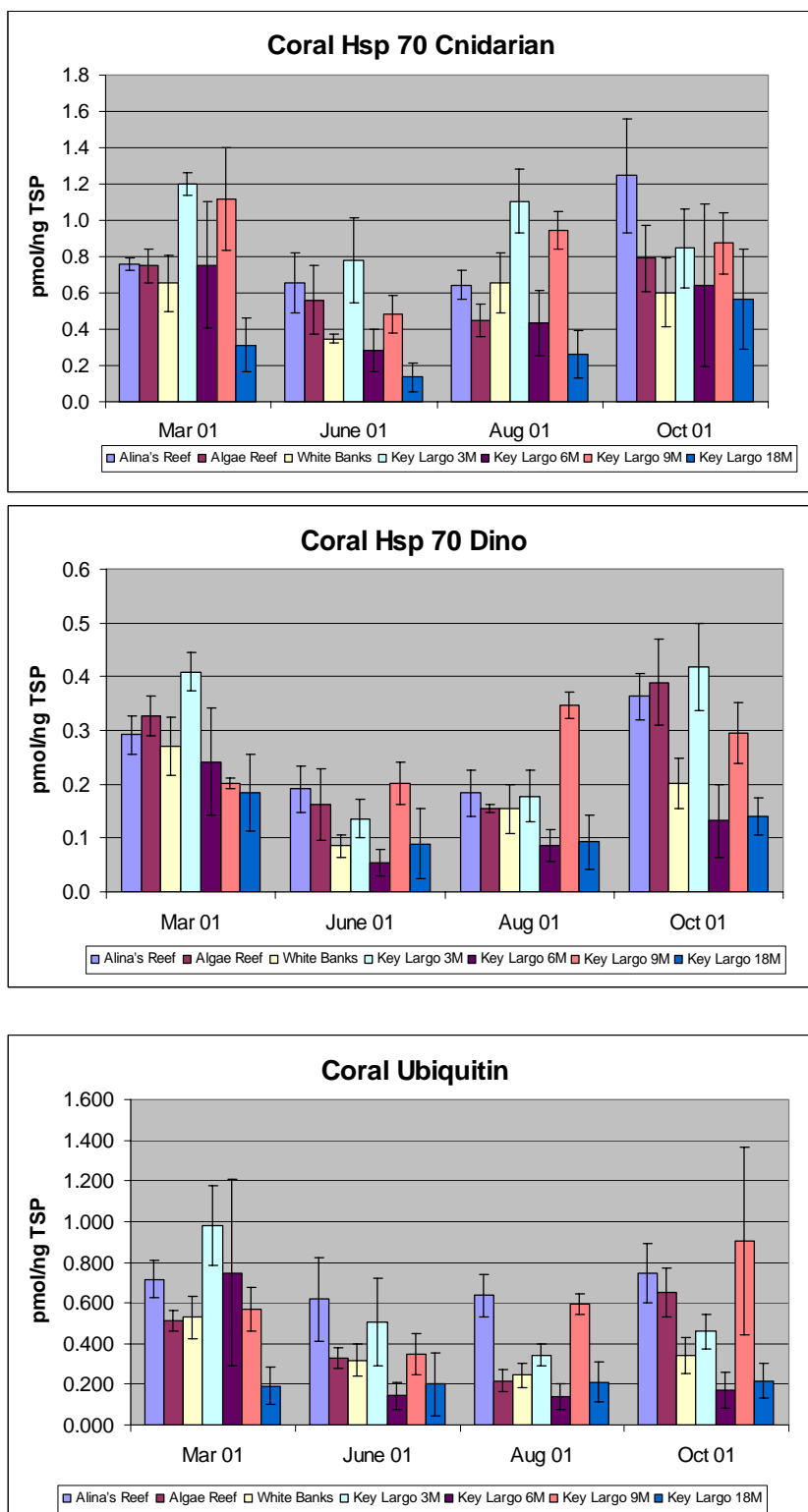


**Figure 6.** Metabolic condition of corals 2001.

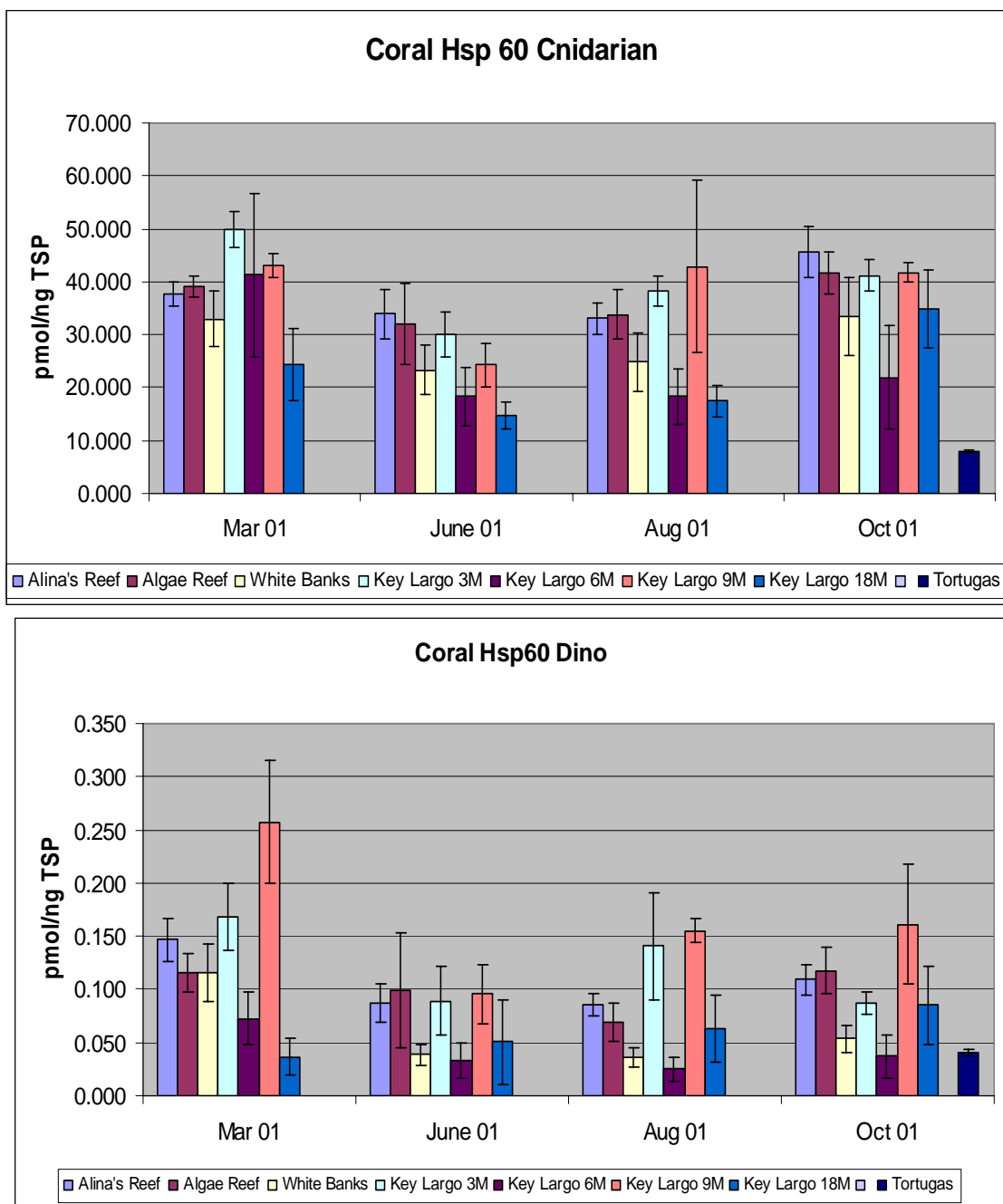


**Figure 6.** Metabolic condition of corals 2001 (cont'd).

## Protein Metabolic Condition

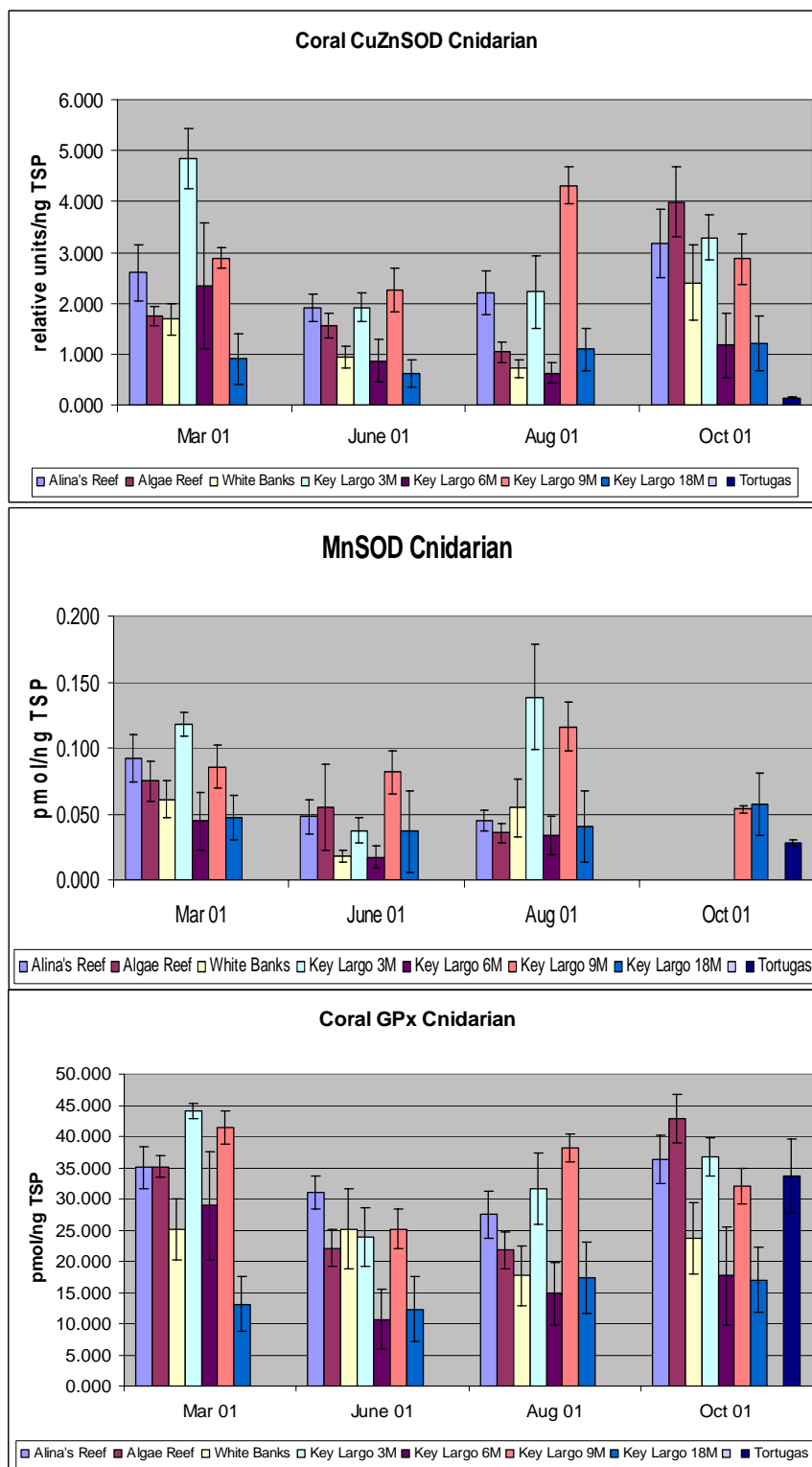


**Figure 7.** Protein metabolic condition of corals 2001.



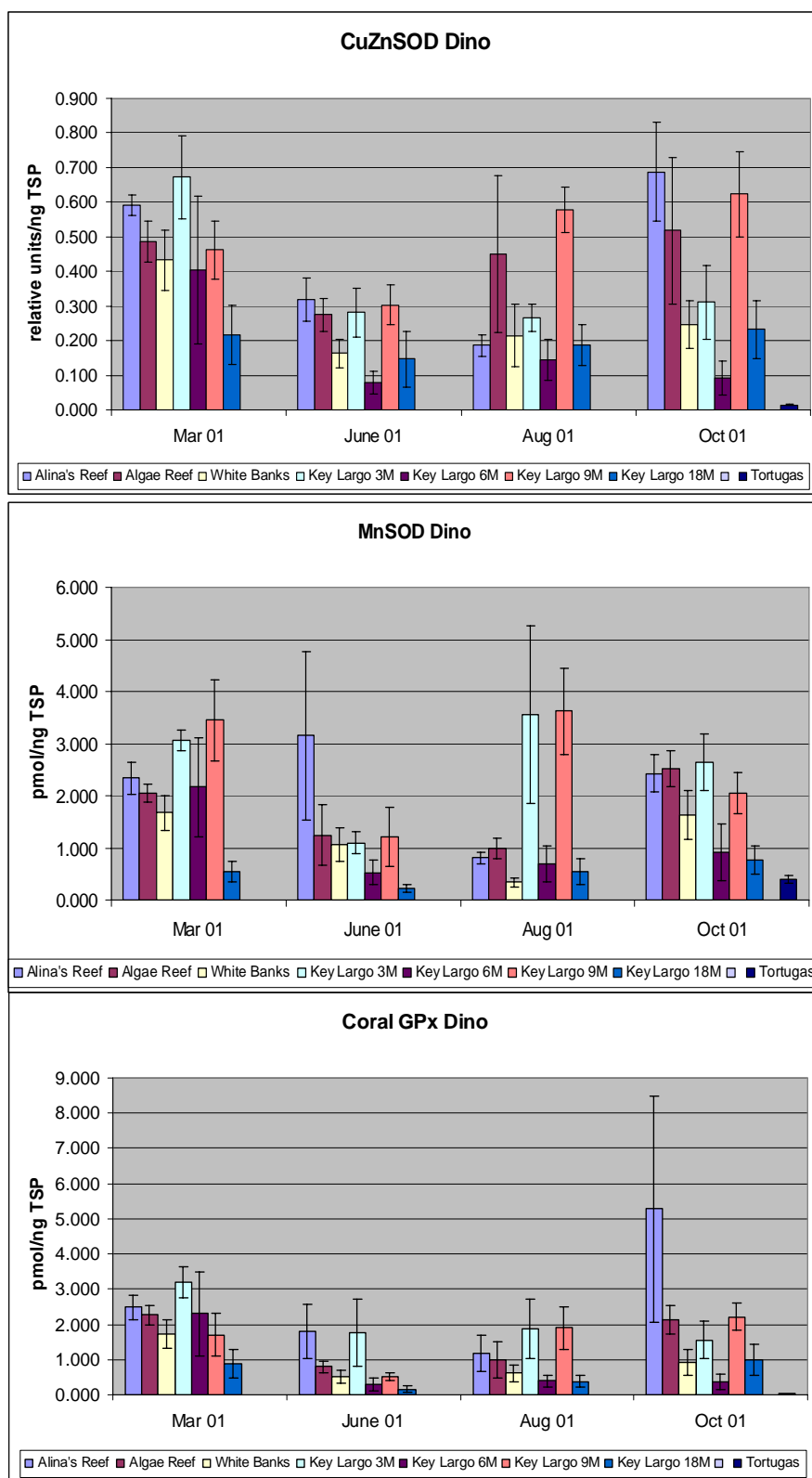
**Figure 7.** Protein metabolic condition of corals 2001 (cont'd).

## Oxidative Stress



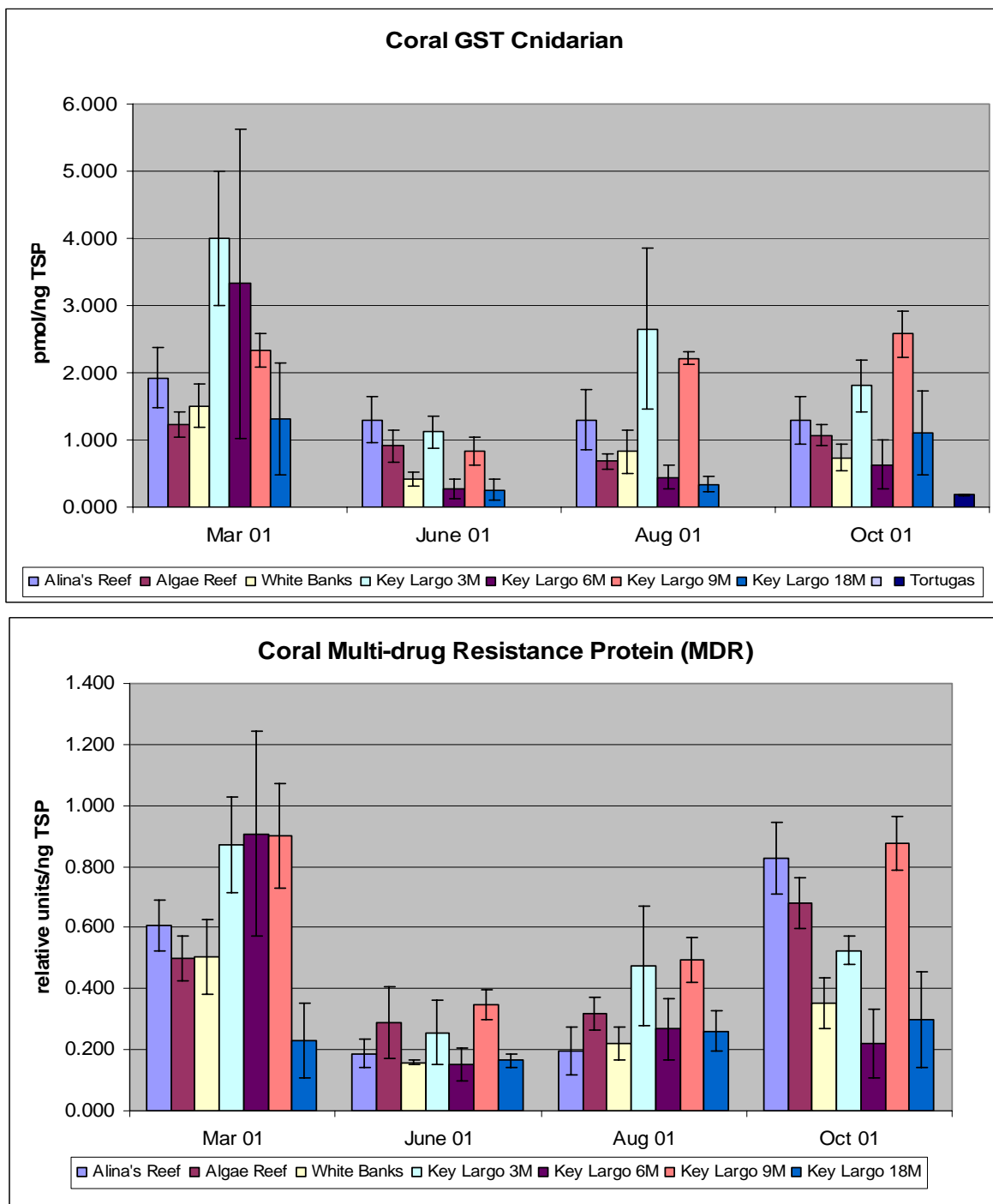
**Figure 8.** Oxidative stress condition of corals 2001.



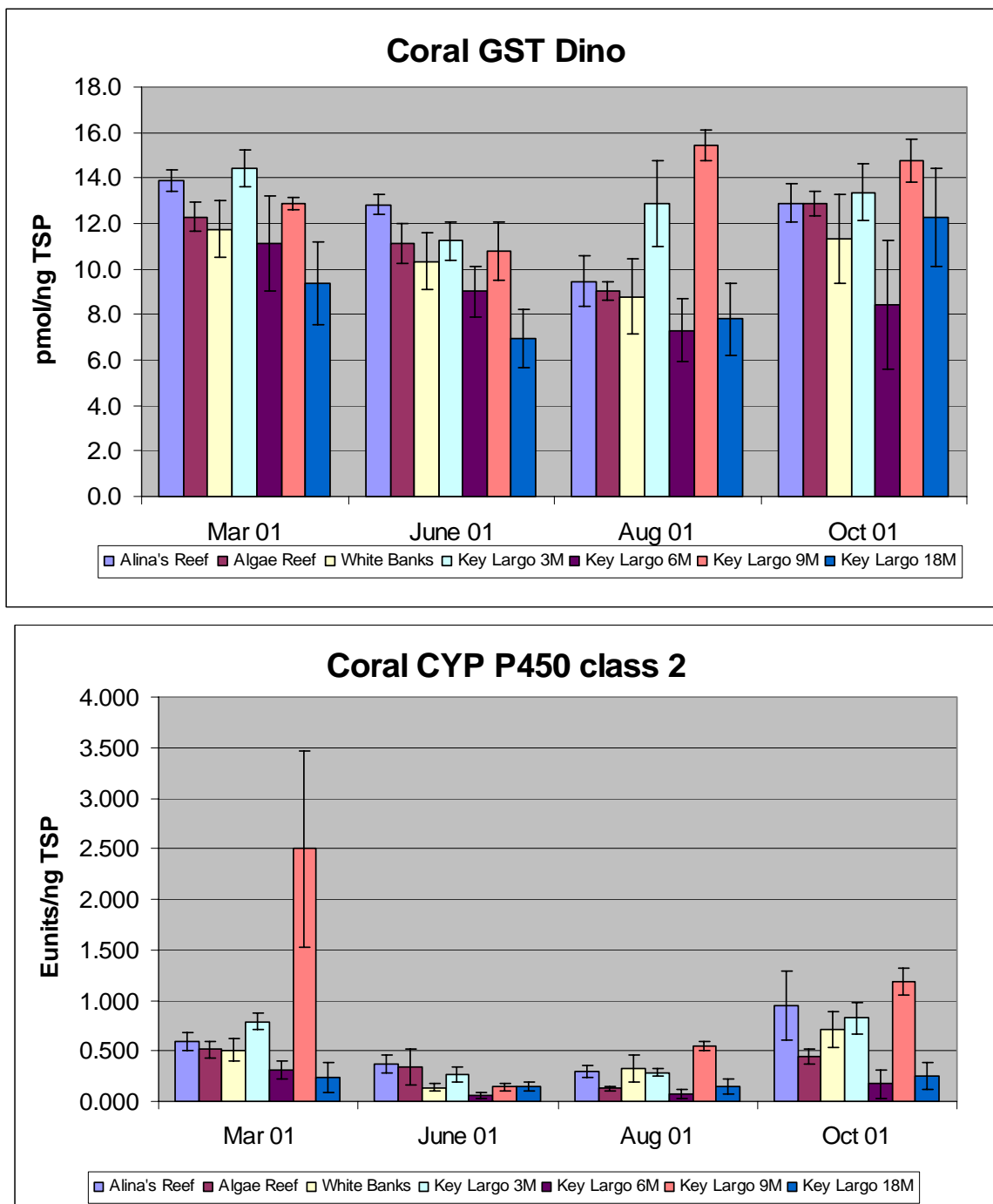


**Figure 8.** Oxidative stress condition of corals 2001 (cont'd).

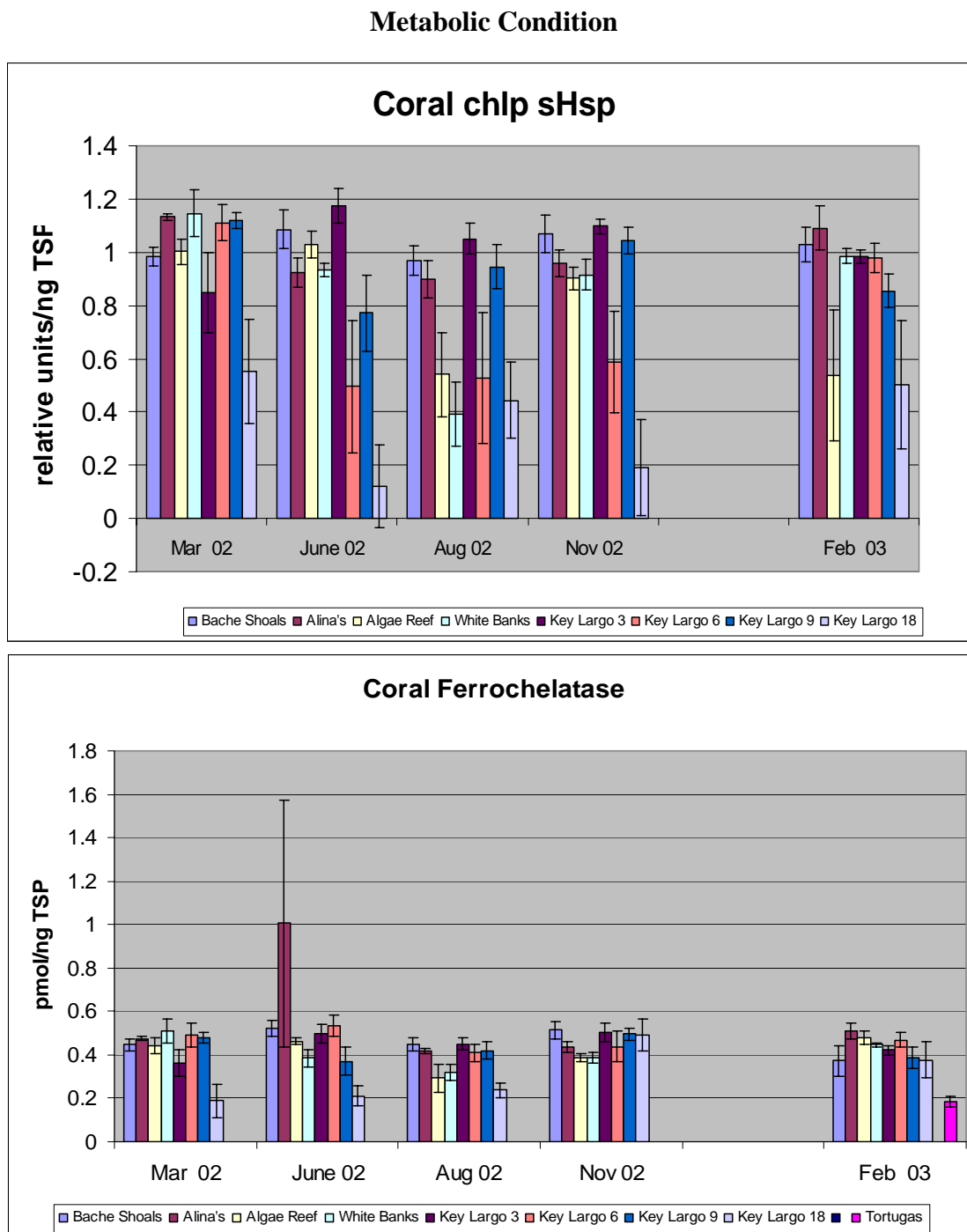
## Xenobiotic Detoxification



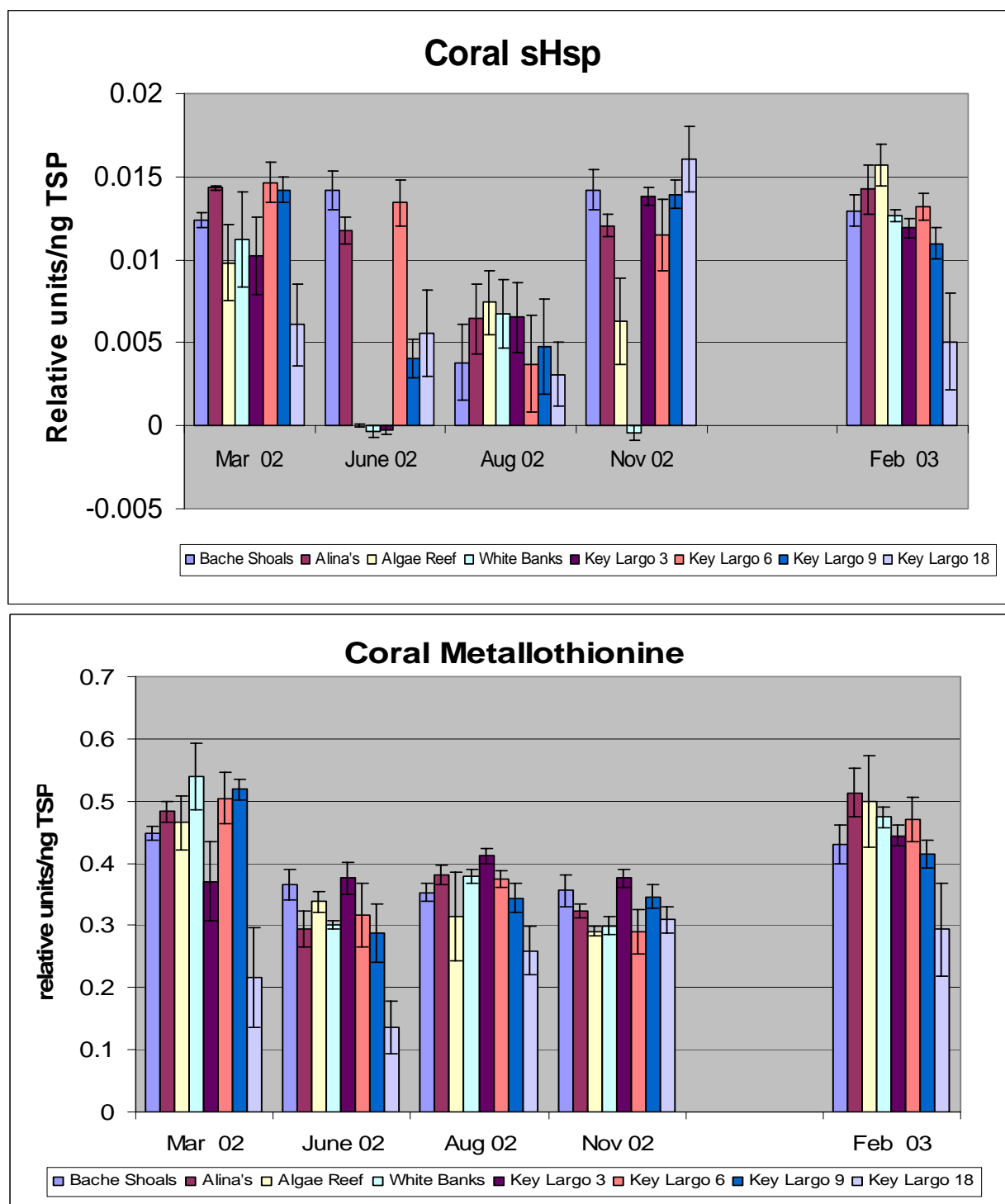
**Figure 9.** Xenobiotic detoxification response in corals 2001.



**Figure 9.** Xenobiotic detoxification response in corals 2001 (cont'd).

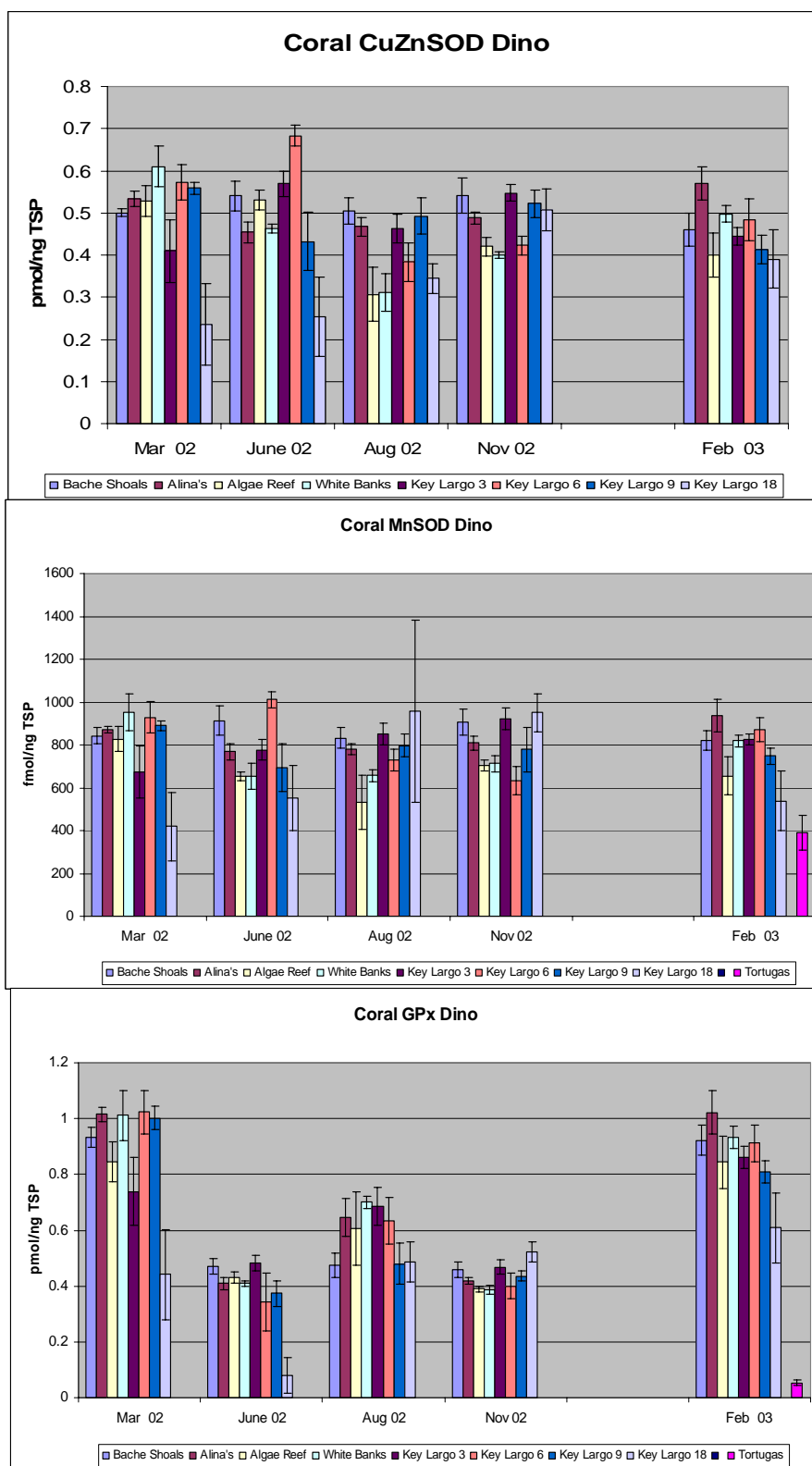


**Figure 10.** Metabolic condition of corals 2002-03.

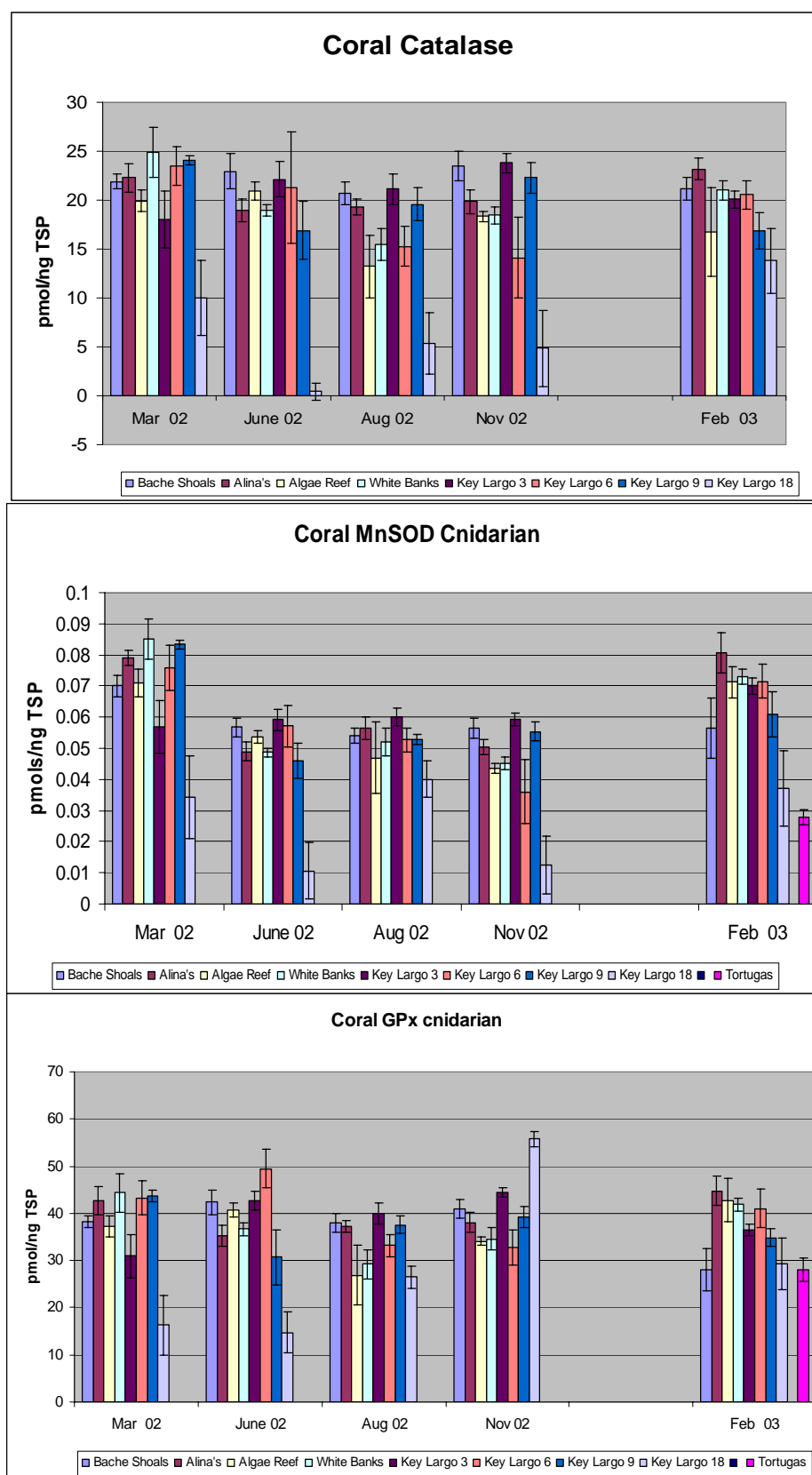


**Figure 10.** Metabolic condition of corals 2002-03 (cont'd).

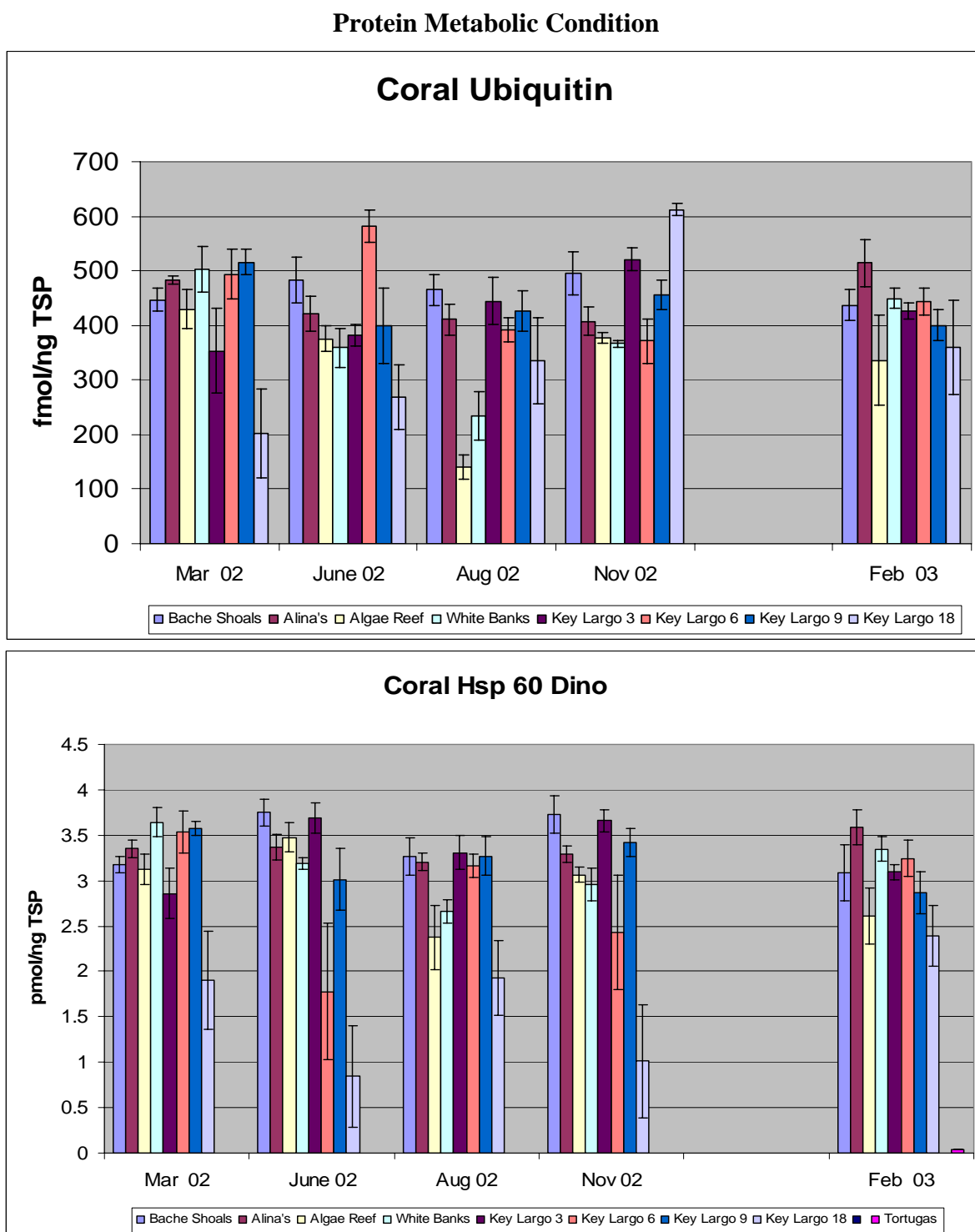
## Oxidative Stress



**Figure 11.** Oxidative stress in corals 2002-03.

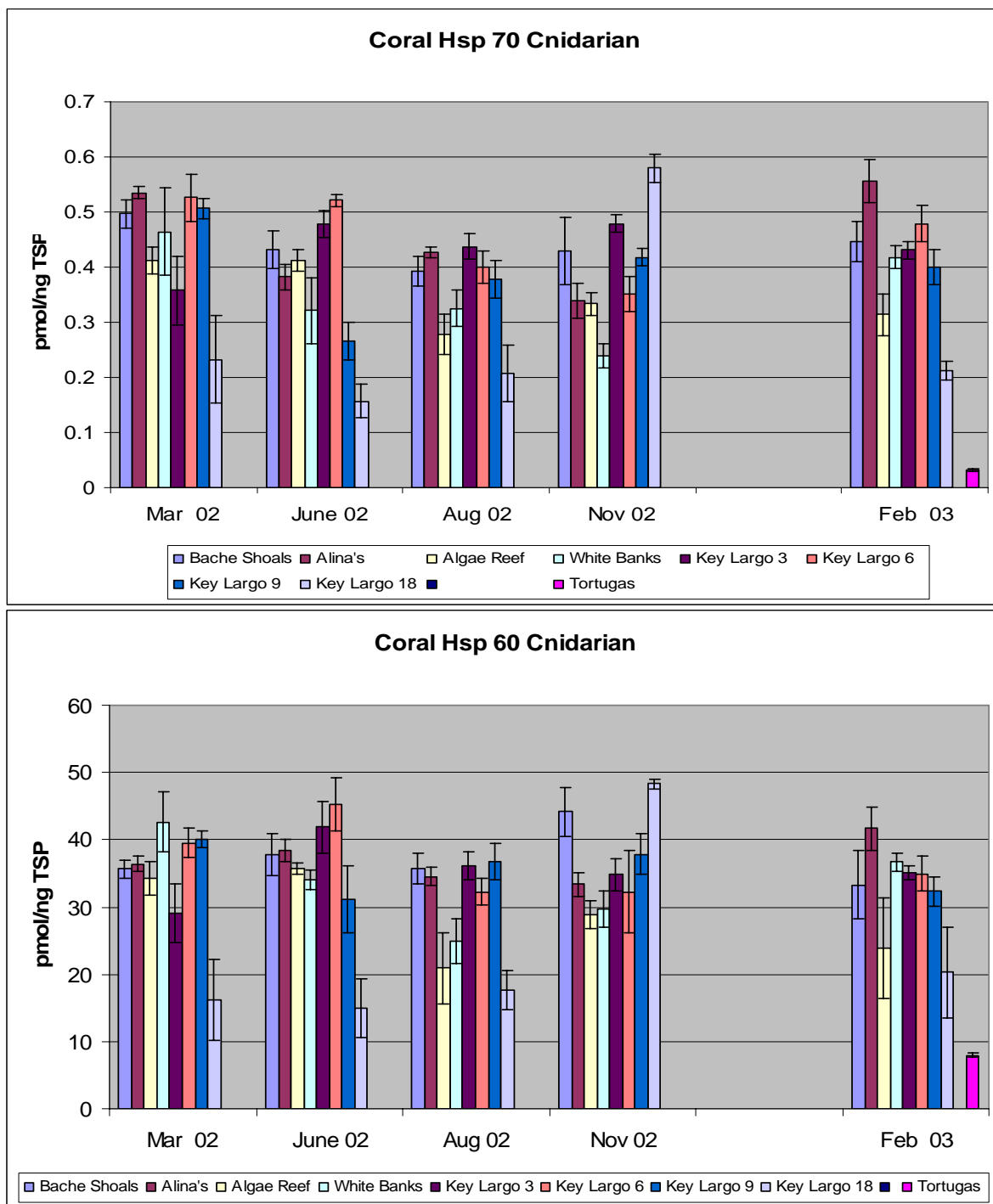


**Figure 11.** Oxidative stress in corals 2002-03 (cont'd).



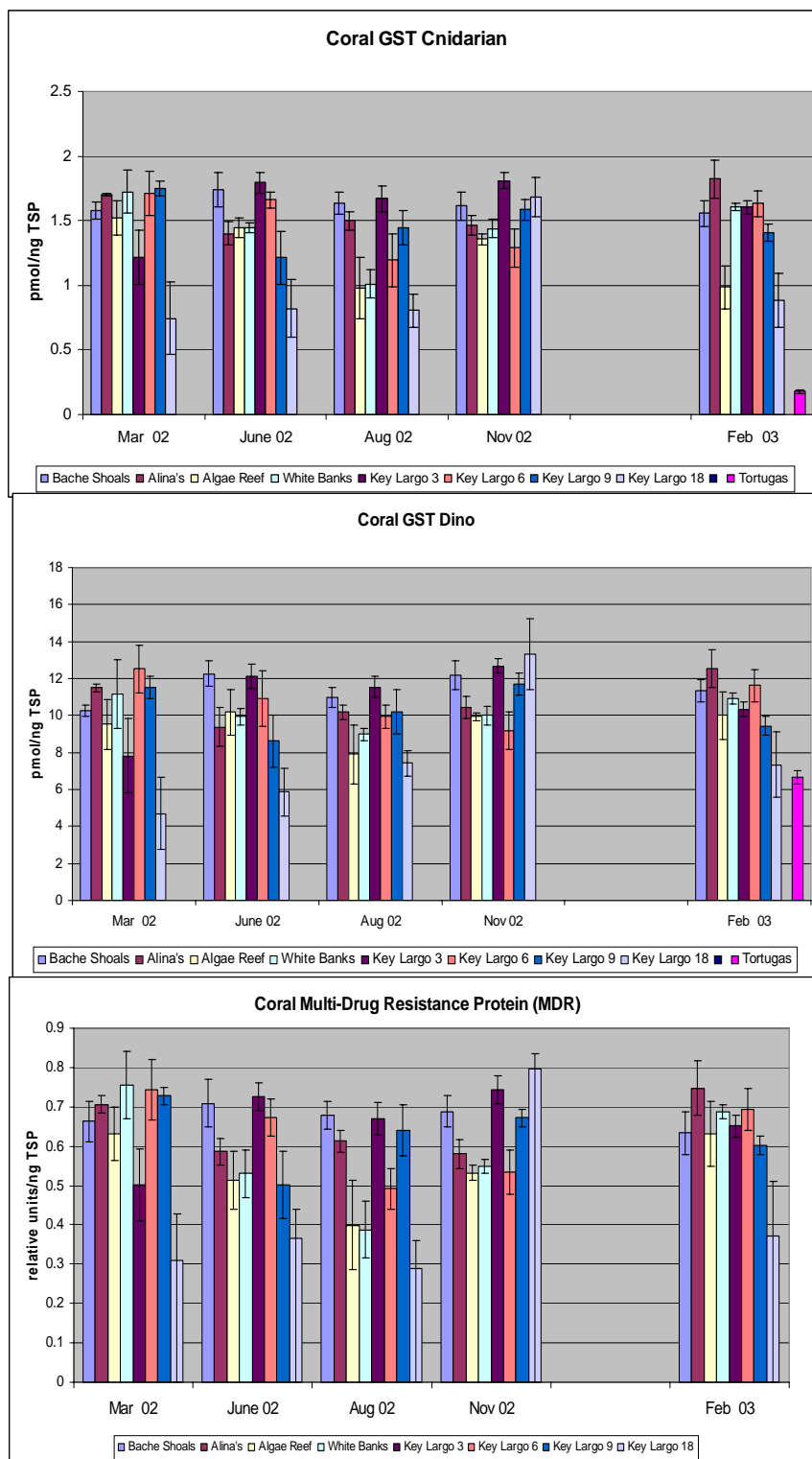
**Figure 12.** Protein metabolic condition of corals 2002-03.



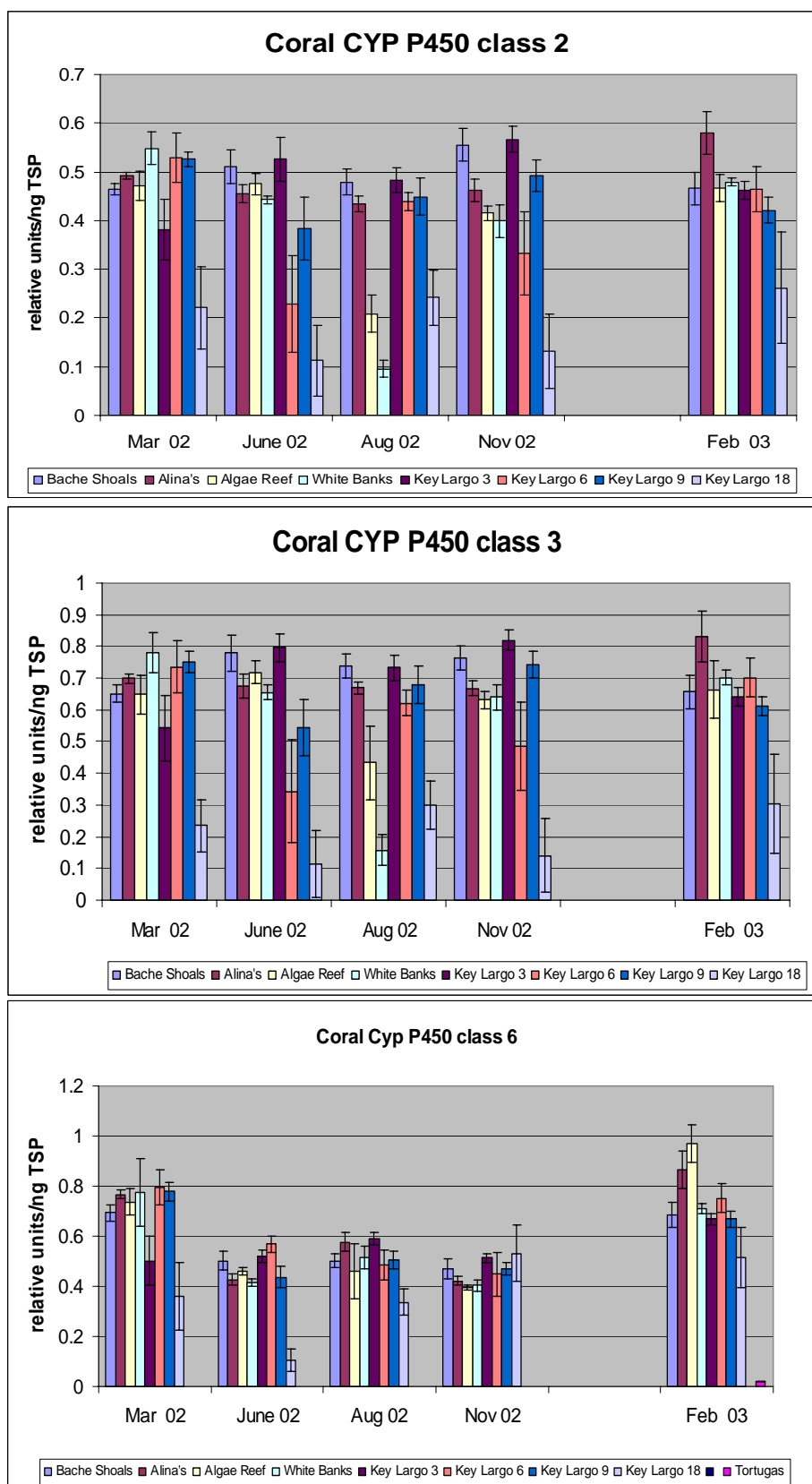


**Figure 12.** Protein metabolic condition of corals 2002-03 (cont'd).

## Xenobiotic Detoxification



**Figure 13.** Xenobiotic detoxification in corals 2002-03.



**Figure 13.** Xenobiotic detoxification in corals 2002-03 (cont'd).

We tested the condition of four subcellular systems that represented metabolic condition, protein metabolic condition, oxidative stress, and xenobiotic exposure. The profiles that were generated provided evidence in support of the efficacy of using CDS in coral health assessment protocols. Our findings provided evidence that corals were responding to different stressors at different locations at different times and further that these were not individual stressors, but rather multiple stressors were responsible for physiological responses being observed. In some locations this meant healthy corals able to cope with the stressors and in other locations, coral death (loss of all living tissue). In general, the coral *Montastraea annularis* at Algae Reef, White Banks Dry Rocks, and the Key Largo 3-m sites appeared to be physiologically stable, but did show increased stress compared to coral located in the Dry Tortugas. In contrast, corals at the two reefs in BNP (Alina's Reef and East Bache Shoals) and at the 6-, 9-, and 18-m sites (sites 2-4) in the FKNMS showed elevated levels of stress and overall poor physiological health. By February 2003, colonies at the 9-m site showed heavy algal overgrowth and two colonies with very little live tissue remaining. At the 18-m site colonies appeared to be dying and one colony had lost all living tissue. The trend over two years of sampling was a general decline at the 9- and 18-m sites (sites 3 & 4) with at least one colony completely dying.

In general, responses varied throughout the year with the winter months (late October through March) appearing more stressful, and with summers less stressful. This is in contrast to 1999, which was a year with unusually high sea surface temperatures. The profiles obtained from 2000 through February 2003, we believe, are reflective of local conditions, and the physiological profiles of each of these trophic levels provide evidence of multiple anthropogenic stressors impacting coral ecosystem health. In developing a pilot prognosis based on these profiles we predict that colonies that are stress-compromised will decline (Key Largo 6- and 18-m), and that colonies at Alina's Reef are declining faster than expected and are subjected to different stressors than other sites. The profiles indicate that colonies at White Banks Dry Rocks are near their tolerance threshold and may experience a rapid change in status and that colonies at Algae Reef and the Key Largo 3-m site should remain healthy.

#### *Objective 4*

The condition of corals at selected sites in Biscayne National Park (BNP) and in the upper Florida Keys National Marine Sanctuary (FKNMS) has been assessed at multiple scales in order to compare the precision, sensitivity, and prognostic capabilities of the CDS with measures traditionally used to assess ecosystem health. Community-scale condition of selected patch reefs was assessed using the well established Atlantic and Gulf Rapid Reef Assessment (AGRRA; Ginsburg et al. 2000). This protocol determines the condition of reefs by evaluating major benthic taxa that comprise them: coral and algae. The condition (i.e., mottling or bleaching) of populations of a key symbiont-bearing foraminiferan (*Amphistegina gibbosa*), living in the vicinity of the corals, is also being monitored according to Hallock et al. (1995). These data will be used to determine if there is a correlation between bleaching stress in the foraminiferan and bleaching or other stress responses in corals. This information will help determine if foraminifera can be used as a surrogate for studies of the mechanisms of coral bleaching. Individual-scale studies include monitoring lesions on corals (Meesters et al. 1997) and the assessment of overall condition (i.e., bleaching, disease, overgrowth, etc.) of the sampled corals. These assessments are compared to measures of health status taken at the cellular physiological level in a coral (*Montastraea annularis*), two fishes (*Haemulon plumieri* and *Stegastes partitus*), an alga

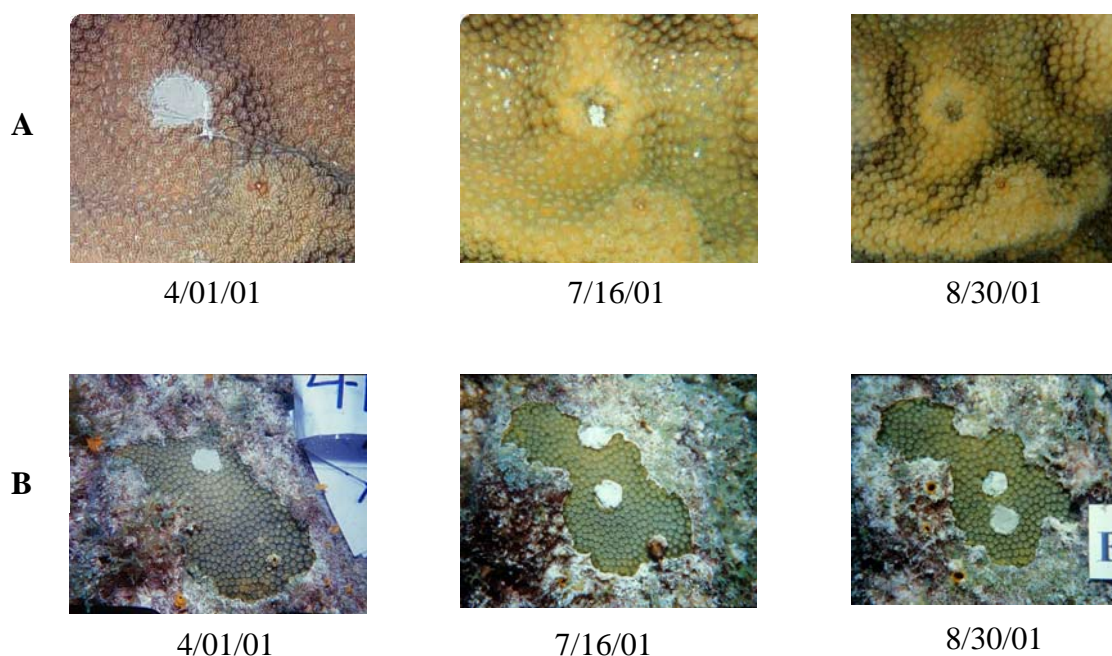
(*Halimeda opuntia*), and a snail (*Coralliophila abbreviata*) using a Cellular Diagnostic System (CDS) (Downs et al. 2000, 2001). The Cellular Diagnostic System assesses indicators of cell integrity indicative of stressed or non-stressed conditions. Environmental data are also being collected, including continuous water temperature measurements (using HOBO data loggers) and nutrient levels (taken at the time of biological sampling), sediment-trap data, and data from other ongoing monitoring studies. The environmental data will be analyzed in conjunction with community, population, coral condition, and molecular data to develop a more comprehensive overview of coral ecosystem health and provide evidence for the underlying stresses.

### *Lesion Regeneration*

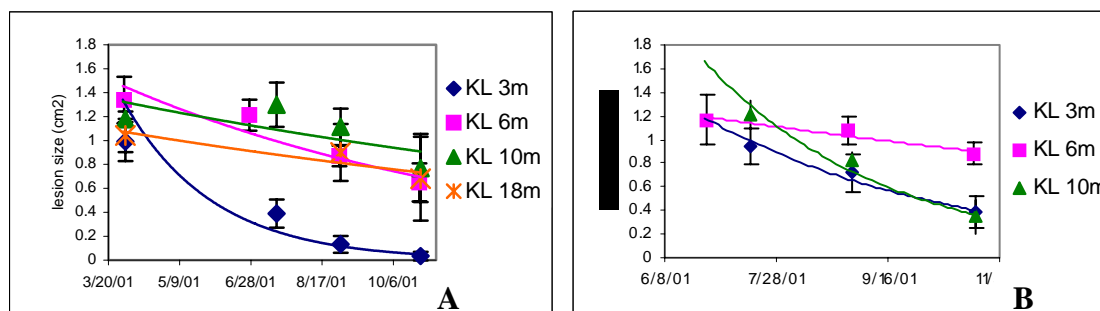
To date, we have compared coral lesion healing with levels of cellular parameters at one site in Biscayne National Park (Alina's Reef) and five sites in the upper Florida Keys National Marine Sanctuary that represent both a depth gradient (3.1-18.3 m) and geographic distribution (3 sites each at 6.1 m depth). To accomplish this, we tagged corals (*Montastraea* complex) that were to be sampled. Corals were sampled using a 1.5-cm punch, removing an approximately 3-mm deep "divot" of tissue from the colony surface. The sampling employed a repeated measures design on a quarterly basis in 2001 and 2002 (March/April, June, August/September, and October/November). The lesions (defined as an area on the colony with no live coral tissue) were monitored by photographing each lesion using a Nikonos V camera with a close-up adapter at each of the quarterly sampling events. Photographs were scanned to digital images, and then the area and perimeter of the lesions were calculated using image analysis software. Tissue samples were analyzed by ELISA for 20 cellular parameters included in the CDS. Our hypothesis was that a coral, which the CDS indicates to be more stressed, would be less likely to regenerate than a coral that CDS indicates to be less stressed.

In March, lesions from the Key Largo 3-m site experienced a large degree of regeneration with some lesions closing completely (Fig. 14A and 15A). Other sites, such as the Key Largo 10-m site, experienced very little regeneration with some lesions showing increases in mortality (Fig. 14B and 15A). However, in June, lesions from the Key Largo 10-m site appeared to regenerate the best, relative to lesions from the shallower corals at the Key Largo 6- and 3-m sites (Fig. 15B). Algae Reef (site 6) and White Banks (site 5) showed the greatest amount of regeneration year round relative to the other two 6-m sites, Alina's Reef and the Key Largo 6-m site, which show very little change year round (Fig. 8A and 8B).

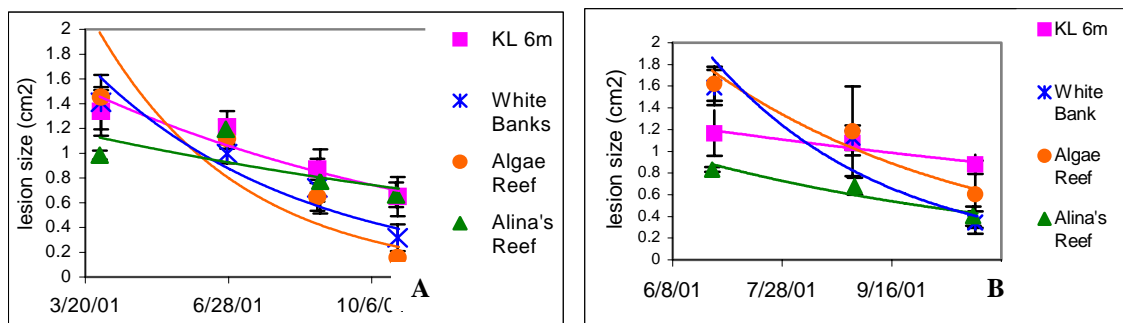
Results of a backward stepwise regression, to determine which of the cellular parameters explained significant variation in coral re-growth, indicated that re-growth was correlated with depth and five of the cellular parameters: MDR (multi-drug resistance protein), dinoflagellate heat shock protein (Hsp) 60, cnidarian Cu/Zn superoxide dismutase, dinoflagellate Mn superoxide dismutase, and dinoflagellate glutathione peroxidase. Corals with high levels of plant Hsp 60 and plant glutathione peroxidase healed more quickly, indicating a healthy status. Lesions in corals with high MDR, cnidarian Cu/Zn superoxide dismutase, and plant Mn superoxide dismutase levels healed more slowly, suggesting they were stressed with a xenobiotic, thus allocating less of their energy to regeneration. These analyses indicate that corals located at Algae Reef showed significantly higher re-growth of lesions than those at the 9- and 18-m sites off of Key Largo.



**Figure 14.** Change in lesion size between March and August 2001: (A) Decrease in lesion size indicating regeneration of lesion at the Key Largo 3-m site. (B) Increase in lesion size indicating increased mortality and algal overgrowth at the Key Largo 9-m site.



**Figure 15.** Change in mean lesion size ( $\text{cm}^2 \pm \text{SE}$ ) along a depth gradient of 3, 6, 10, and 18 m in Key Largo. Lines fitted to an exponential model. (A) March sampling lesion (B) June sampling lesion.

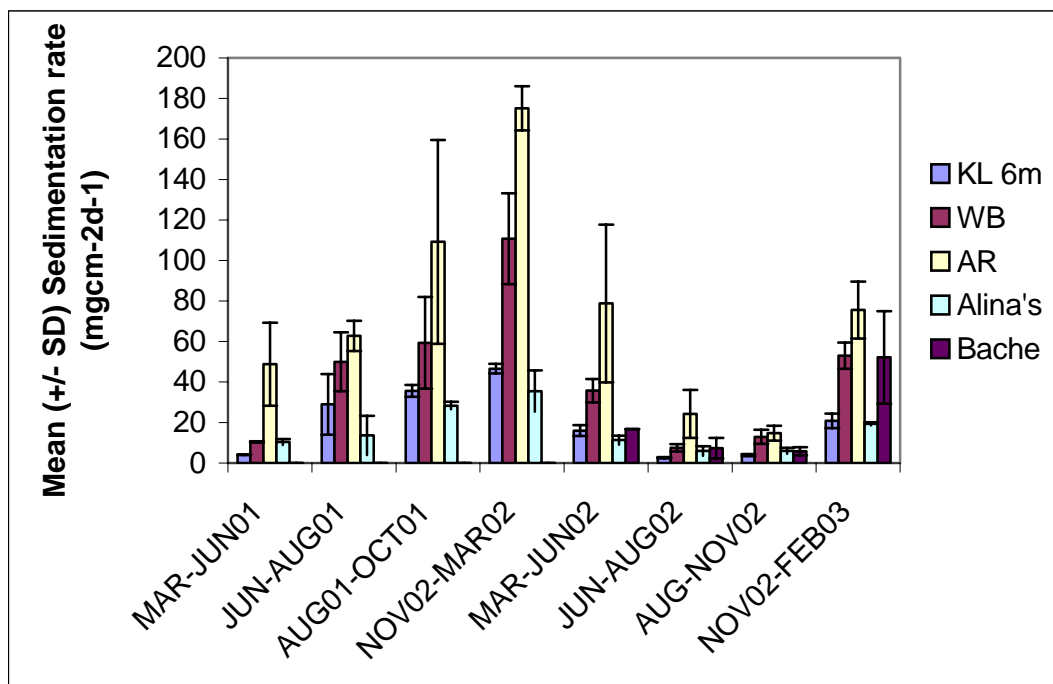


**Figure 16.** Change in mean lesion size (cm<sup>2</sup> ± SE) for the four 6-m sites including Key Largo 6-m (site 2), White Banks (site 5), Algae Reef (site 6), and Alina's Reef (site 7). Lines fitted to an exponential model. (A) March sampling lesion (B) June sampling lesion.

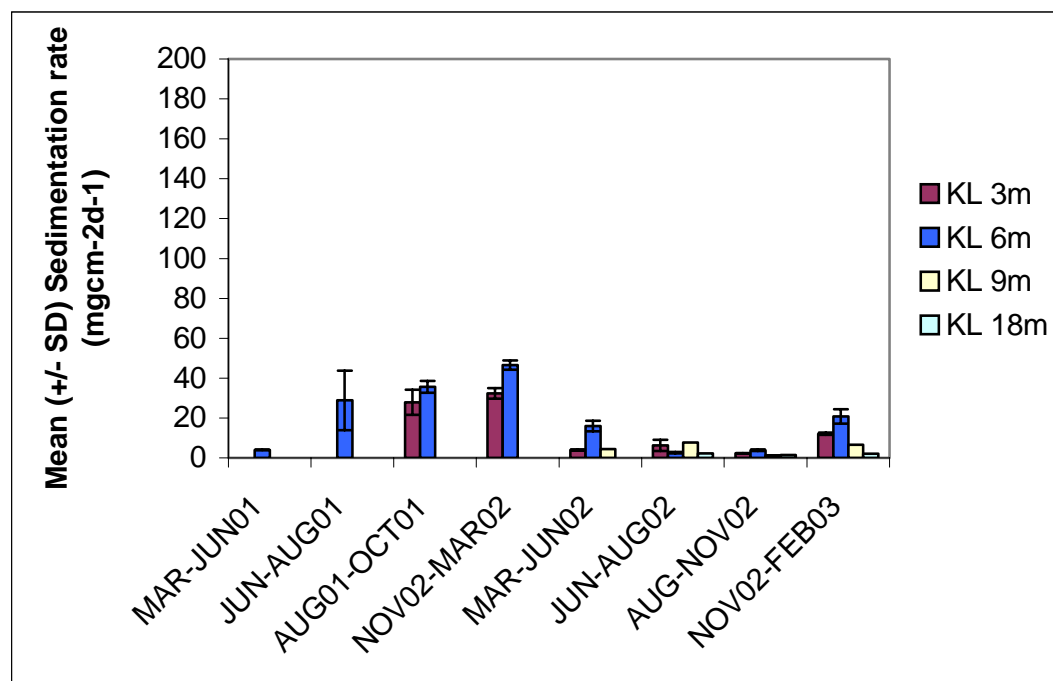
Sedimentation rates were highest at Algae Reef (site 6) and White Banks (site 5) throughout 2001-2002 (Fig. 17). Sedimentation rates were higher in 2001 than 2002 with the highest rates in the winter months (Fig. 17 and 18). Sedimentation data for the Key Largo 9- (site 3) and 18-m (site 4) sites was only collected in 2002 and during that year all sites along the Key Largo depth gradient had very low sedimentation rates (Fig. 18).

With the exception of June to August 2002, Algae Reef and White Banks had the highest regeneration rates of the 6-m sites between 2001 and 2002 (Fig. 3). Alina's Reef (site 7) and the Key Largo 6-m site (site 2) generally had low regeneration rates, but showed variability among colonies and seasons (Fig. 19). Regeneration rates were higher in 2001 than in 2002 for most sites, except for White Banks, which showed little change (Fig. 19). Seasonality was observed in regeneration with the winter months tending to have the lowest regeneration rates (Fig. 19). Sedimentation positively correlated with regeneration in 2001, and the 6-m sites, which had the highest sedimentation rates (Algae Reef, White Banks), also had the highest regeneration rates. Positive trends were still observed in 2002, but were no longer significant.

No depth trends were observed in regeneration in either 2001 or 2002. The Key Largo 3-m site (site 1) had relatively high regeneration rates throughout 2001-2002 with a significant increase in regeneration in 2002 (Fig. 20). The Key Largo 6-m site had relatively low regeneration rates throughout 2001-2002 (Fig. 20 and 21). High variability in regeneration among seasons and among colonies was observed at the Key Largo 9- and 18-m sites with some colonies showing high increases in mortality and other colonies showing the ability to regenerate lesions (Fig. 20 and 21).

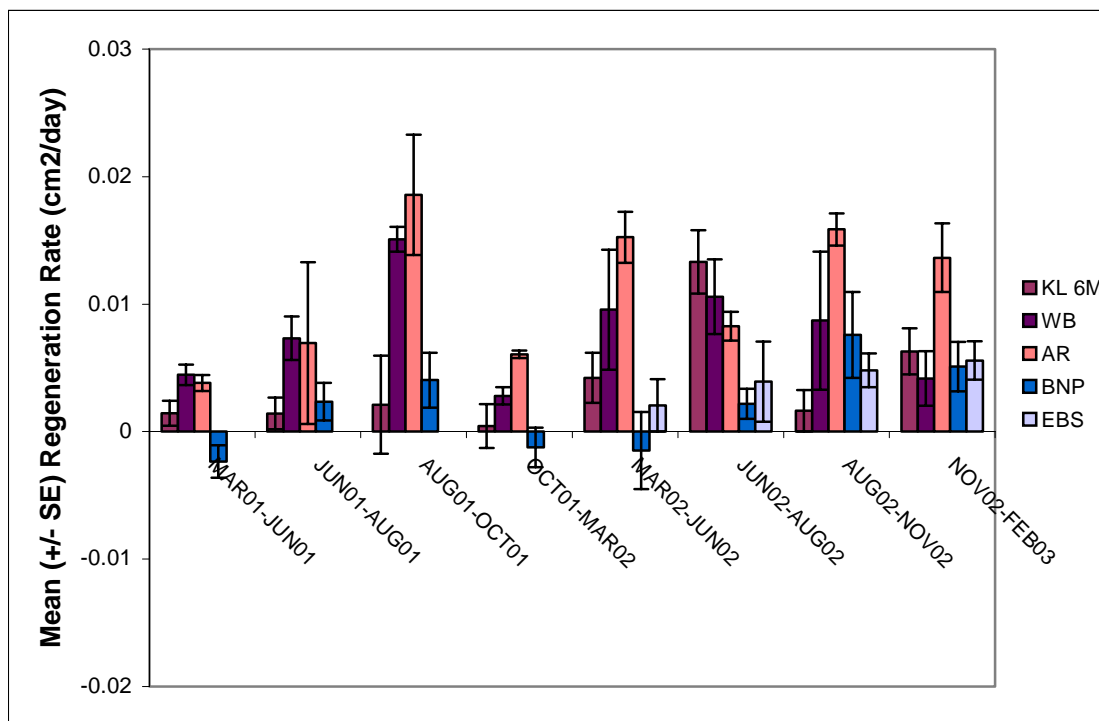


**Figure 17.** Mean sedimentation rates at 6-m sites, 2001-2003. Error bars represent standard deviations.

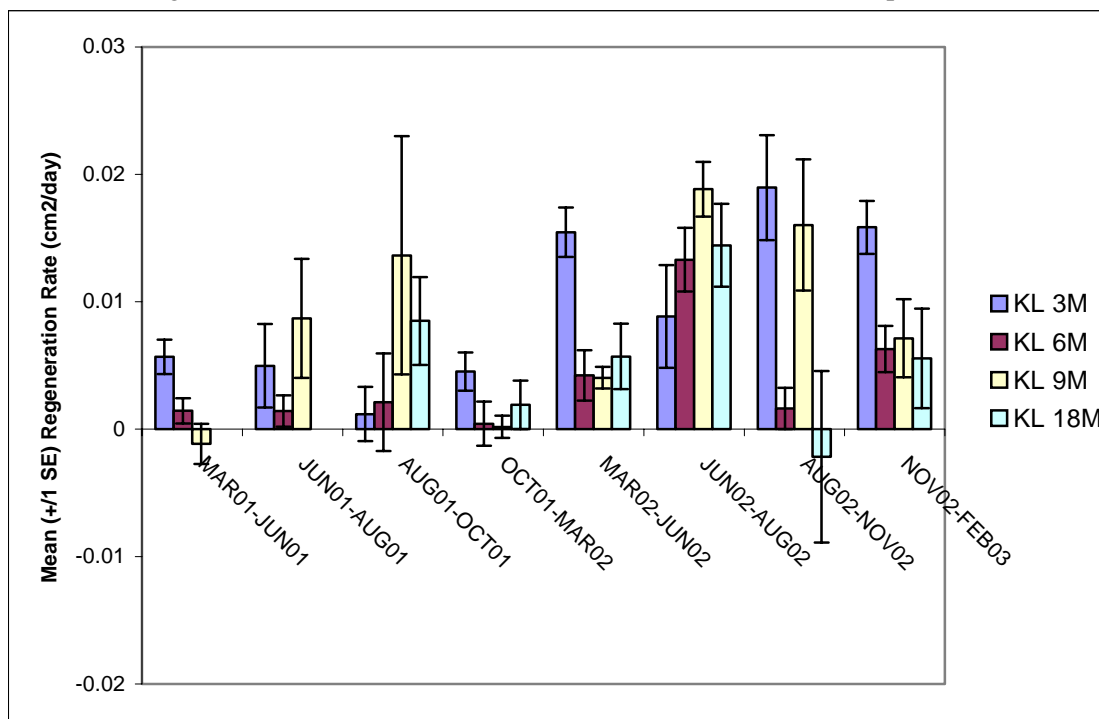


**Figure 18.** Mean sedimentation rates along Key Largo depth gradient, 2001-2003. Error bars represent standard deviations.

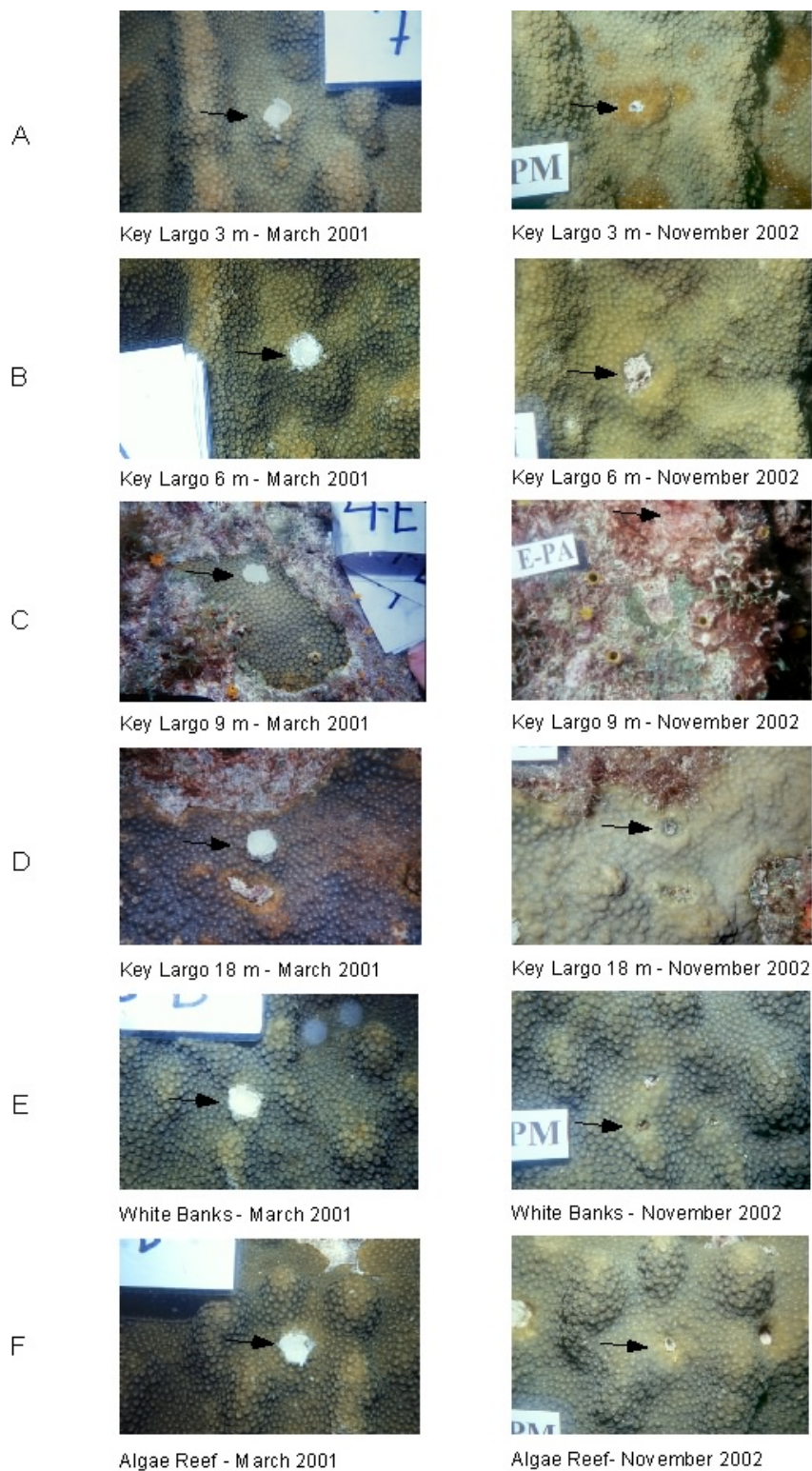




**Figure 19.** Mean regeneration rates at 6-m sites from 2001 to 2003. Error bars represent standard errors.



**Figure 20.** Mean regeneration rates along Key Largo depth gradient from 2001 to 2003. Error bars represent standard errors.



**Figure 21.** Sample pictures showing changes in lesion size between March 2001 and November 2002 at Sites 1-6: (A, D-F) Decrease in lesion size indicating regeneration of sampling lesion; (B) Little change in lesion size; (C) Complete loss of live tissue and algal and sponge overgrowth.

### *Objective 5*

Throughout this project, we have welcomed the participation of individuals from many walks of life. We have had divers participate on various missions including: individuals from a local high school marine biology class, retirees from the local community, resource managers, graduate student volunteers, and industry. Through these interactions, we have been able to communicate with and educate others about the novel technology we are testing, the similarity of this technology to modern biomedicine, and the prospects that this technology brings to understanding coral reef degradation and development of science-based strategies to combat them. We have also engaged the scientific and resource management community in evaluating and critiquing our data and experimental design through a recent workshop (March 15, 2002). We had representatives from academia, the State of Florida, Biscayne National Park, USGS, Florida Keys National Marine Sanctuary, National Undersea Research Program, industry, Mote Marine Laboratory, and the National Ocean Service. They were able to review our data and provide critical input to our second-year design. Two significant recommendations from the meeting were to increase the spatial scale of the project and conduct laboratory challenge experiments with suspect stressors.

In January 2004, we held our final constitutive workshop for this project. Over 40 representatives from academia, non-profit organizations, and industry as well as local, state, and federal agencies attended. Our findings were well received and valuable constructive criticism was given by the participants. In summary the participants agreed that the technology was valuable and did show promise for providing useful information for conducting coral reef health assessment and had the capability of helping elucidate the “drivers” in coral reef system condition and response. We were encouraged to continue development of the CDS technology, specifically focusing on developing sound linkages between a specific stressor and a unique profile of physiological response, and the fate of the organism or population to exposures analogous to a forensic investigation that links the “victim, the smoking gun, and the bullet.”

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